PLANT COMPETITIVE INTERACTIONS UNDER SOIL NUTRIENT TRANSPORT LIMITATION

Dissertation

zur

Erlangung der naturwissenschaftlichen Doktorwürde

(Dr. sc. nat.)

vorgelegt der

Mathematisch-naturwissenschaftlichen Fakultät

der

Universität Zürich

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Zürich, 2016

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Summary

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Plant competitive interactions play a major role in plant species co-existence. The niche concept and the theory of resource partitioning can explain the co-existence of species in plant communities by prevention of competitive exclusion. The resource partitioning theory suggests that organisms can reduce resource pools to concentrations that are too low for others to persist, and the trade-off between the resource specific minimum requirements of species can explain their co-existence. However, the theory was tested experimentally on planktonic algae in an aquatic environment and may provide only a limited explanation for resource competition between plants in terrestrial environments. The ability of plants to reduce concentrations of a resource pool in soils is limited because resource preemption is generally locally restricted. Nutrient mobility has been proposed to affect plant resource preemption and therefore, the competitive strength between plant individuals. I tested this theory in two glasshouse experiments. I manipulated the ratio of mobile to immobile mineral nitrogen forms in soils using the commercial nitrification inhibitor DMPP (3, 4-dimethyl pyrazole phosphate) (**Chapter 1**) in a pot experiment. DMPP was applied to plant communities containing individuals of one, two or four species and further individuals of each species were grown without neighbors. I monitored plant growth by sequential size trait measurements and tracked nutrient movement between plants using ^{33}P, ^{32}P and ^{15}N nutrient tracers. Aboveground biomass was analyzed at both the individual and community level. DMPP had consistently positive effects on plant growth but there were no indications for reduced competitive interactions. In contrast, the evenness of communities was reduced. These results suggest that nutrient mobility was less important for competition under increased productivity in this experiment. In a second experiment I adapted the experimental design from the previous study but manipulated soil nutrient mobility by disconnecting plants from a common mycorrhizal network (CMN) (**Chapter 2**). I found that the disconnection from CMNs reduced aboveground biomass

considerably whereas belowground biomass increased. I could not find evidence for reduced competition but complementarity between species did decrease with the treatment. These results suggest that nutrient mobility was less important for competitive interactions than any negative effects of CMN disconnection on plant growth potentially caused by the prevention of complementary resource use.

Plant competitive interactions decrease with distance to their neighbors and restricted range of seed dispersal and clonal growth result in aggregates of conspecifics and consequently more frequent intraspecific than interspecific interactions in plant communities. Spatial aggregation of conspecifics has been proposed to prevent competitive exclusion as inferior species are protected from interactions with superior competitors. However, spatial aggregation cannot result in stable co-existence as aggregate edges can be invaded. "Heteromyopia" has been suggested as a mechanism to stabilize co-existence in spatially structured plant communities through the differentiation of competition distances between heterospecifics and conspecifics. Heterospecifics supposedly compete more over short distances whereas conspecifics compete proportionally more over longer distances. I tested this widely unexplored theory empirically in a glasshouse and a field study (**Chapter 3**). I measured uptake of ^{33}P and ^{15}N nutrient tracer by con- and heterospecific plant individuals at increasing distances from a labelled focal individual. In both studies results varied between nutrients and plant species but nutrient uptake at close proximity was generally greater in heterospecifics. Nutrient uptake in conspecifics decreased linearly with distance whereas nutrient uptake in heterospecifics had greatest declines at close proximity. These results do not allow me to reject the concept of heteromyopia but highlight the need for consideration of species specific distance dependencies in competitive interactions.

The studies presented here show empirical tests for two hypothetical mechanisms that have been suggested to affect plant competitive interactions. Our results give insights into the

relative importance of the tested mechanisms and their potential impact on plant community structure.

Zusammenfassung

Pflanzenkonkurrenz spielt eine wichtige Rolle für die Koexistenz von Arten. Das Konzept der ökologischen Nische sowie die Theorie der Ressourcenaufteilung können Koexistenz erklären. Laut Ressourcenaufteilungstheorie können Organismen die Nährstoffkonzentration in ihrer Umgebung reduzieren und damit andere Arten mit höheren Nährstoffansprüchen verdrängen. Ein trade-off von nährstoffspezifischen Minimalanforderungen aller Arten ermöglicht ihre Koexistenz. Diese Theorie wurde an Planktonalgen und somit in Wasserökosystemen getestet und kann nur bedingt auf terrestrische Ökosysteme angewendet werden, da die Möglichkeit von Pflanzen die Nährstoffkonzentration im Boden zu beeinflussen lokal begrenzt ist. Nährstoffmobilität könnte beeinflussen inwieweit Pflanzen eine Nährstoffkonzentration herabsetzen und somit potentiell mit anderen konkurrieren können. Ich testete diese Theorie in zwei Gewächshausexperimenten. Mithilfe des auch kommerziell genutzten Nitrifikationsinhibitors DMPP (3,4-Dimethylpyrazolphosphat) veränderte ich das Verhältnis von mobilen zu immobilen Stickstoffformen im Boden (**Kapitel 1**). Der Inhibitor wurde auf Pflanzengesellschaften mit einer, zwei oder vier Arten angewendet wobei jede Art auch ohne Konkurrenz wuchs. Ich zeichnete das Pflanzenwachstum mit wiederholten Größenmessungen auf und verfolgte die Nährstoffbewegung zwischen den Pflanzen mithilfe von ^{33}P, ^{32}P und ^{15}N Nährstoffisotopen. Auf oberirdischer Biomasse basierende Daten wurden unter Berücksichtigung von Selektions- und Komplementaritätseffekten sowie Ebenheit und Größenungleichheit der Gesellschaft auf Individuen- und Gesellschaftsebene analysiert. DMPP hatte generell positive Effekte auf das Pflanzenwachstum, es gab jedoch keine Hinweise auf reduzierte Pflanzenkonkurrenz. Im Gegenteil war die Ebenheit der Gesellschaften reduziert. Diese Resultate suggerieren, dass bei erhöhter Produktivität, wie in diesem Experiment, die Nährstoffmobilität für die Konkurrenz unwesentlich war. Das zweite Experiment basierte im Design auf dem Vorangegangen, jedoch reduzierte ich hier die Nährstoffmobilität indem ich

die Pflanzen von einem gemeinsamen Mykorrhizennetzwerk (GMN) trennte (**Kapitel 2**). Durch die Trennung vom GMN nahm das oberirdische Pflanzenwachstum ab, während das Wurzelwachstum zunahm. Es gab keine Hinweise auf Veränderungen der Konkurrenzbeziehungen zwischen den Pflanzen, aber die Komplementarität zwischen Arten wurde verringert. Nährstoffmobilität war demnach weniger bedeutsam als die negativen Konsequenzen einer Trennung vom GMN, potentiell beruhend auf verminderter komplementärer Ressourcennutzung.

Konkurrenz zwischen Pflanzen nimmt mit Distanz ab und räumlich beschränkte Saatverbreitung sowie klonales Wachstum resultieren in räumlicher Aggregierung von Individuen einer Art, womit intraspezifische Konkurrenz in Pflanzengesellschaften verbreiteter ist als interspezifische. Räumliche Aggregierung könnte zwar den Ausschluss untergeordneter Arten verzögern, da diese vor Interaktionen mit starken Konkurrenten geschützt sind, jedoch nicht zu stabiler Koexistenz führen, da Konkurrenten letztendlich über Aggregatränder eindringen können. ,,Heteromyopia“ ist ein Mechanismus, der durch die Differenzierung von Konkurrenzdistanzen zwischen konspezifischen und heterospezifischen Arten Koexistenz stabilisieren könnte. Heterospezifische Arten würden demnach überwiegend über kurze Distanzen konkurrieren während die Konkurrenz mit konspezifischen Arten vermehrt bei größeren Distanzen aufträte. Ich testete diese weitgehend unerforschte Theorie in einer Feld- und einer Gewächshausstudie, in der ich die Aufnahme von ^{33}P und ^{15}N Nährstoffisotopen in Pflanzen mit zunehmender Entfernung von einer markierten Zentrumspflanze maß (**Kapitel 3**). Die Resultate beider Studien zeigten Unterschiede zwischen Nährstoffen und Arten, wobei die Nährstoffaufnahme bei kurzen Distanzen und heterospezifischen Arten generell größer war. Die Nährstoffaufnahme von heterospezifischen Arten nahm außerdem schon nach kurzer Distanz stark ab, während die Aufnahme bei konspezifischen Arten linear mit der Distanz abnahm. Diese Resultate lassen keinen Ausschluss von Heteromyopia zu und machen deutlich, dass artspezifische Distanzabhängigkeiten von Konkurrenz berücksichtigt werden sollten.

Die hier präsentierten Studien bieten empirische Belege für zwei Mechanismen, die potentiell Pflanzenkonkurrenz beeinflussen. Außerdem geben uns die vorliegenden Ergebnisse Hinweise auf die Bedeutung der getesteten Mechanismen und deren potentielle Effekte auf die Struktur von Pflanzengesellschaften.

Introduction

General Introduction

The role of competition

The fast-paced species loss that ecosystems currently face is estimated to be four orders of magnitude greater than background extinctions (Barnosky et al. 2011) and can be related to anthropogenic habitat degradation (Naeem et al. 2012). The great goal of our time is to halt diversity loss and rebuild ecosystems that are already degraded (Rio de Janeiro Earth Summit 1992). The consequences of species loss and the importance of biodiversity for ecosystem processes and functioning are the focus of numerous studies in ecology (Hooper et al. 2005, 2012, Cardinale et al. 2012). Biodiversity is driver of ecosystem productivity, resistance, resilience and stability and has been identified as fundamental for human well-being (Millennium Ecosystem Assessment 2005, Isbell et al. 2011, Cardinale et al. 2012, Hooper et al. 2012) by providing food security, fresh water and regulating services that reduce the impact of environmental change. A driving force behind the positive effects of biodiversity on ecosystem processes and functioning is the ability of more diverse organisms to use available resources complementarily (Hector and Hooper 2002, Silvertown 2004). Contemporary research is uncovering the major threats of habitat degradation and biodiversity loss and highlights the need for a better understanding of the mechanisms that drive co-existence. Of these, plant competition has been identified as a major determinant of species co-existence (Gause 1934) and consequently the diversity of plant communities.

In 1917, Sir Arthur Tansley reported the first experiment on plant competition to the British Ecological Society (Keddy 2001). Long before Tansley carried out this experiment, Darwin had already identified 'the struggle between individuals' as the major force connecting organisms in living systems. Darwin was aware that each individual requires a certain range of conditions and set of resources which can maximise its fitness, or allow it to persist, grow and reproduce until one of these conditions or resources is limiting, as later formulated in Liebig's

minimum law (1928). Competition occurs once individuals require the same limiting resource (Hutchinson 1957). Depending on their competitive strength, negative effects of competition on the individuals involved may be more or less severe. What follows in cases of large differences in competitive strength is the exclusion of weak competitors from a community by relatively stronger competitors (Gause 1934). Competitive exclusion is a naturally occurring process, which makes it seem all the more paradoxical when similar competitors co-exist. The paradox of co-existence has been a subject of ecological research and heated debates between scientists for centuries (Aarssen 1983; Grubb 1977; Hutchinson 1957; Chesson 2000).

Co-existence theory and resource partitioning

Experiments and the observation of natural communities have contributed to our current understanding of competition and the avoidance of competitive exclusion in highly diverse communities. The concept of the ecological niche provides a theoretical framework for the co-existence of species and is widely accepted as an explanation for the avoidance of competitive exclusion. The "niche" was first mentioned in 1917 (Grinnel 1917) and has been refined since (Chase and Leibold 2003). Originally a species' niche was referred to as its habitat and food requirements but later works have adapted this view. Hutchinson (1957) described the niche as "n-dimensional hypervolume" which illustrates the increasing complexity of factors that are considered, including abiotic and biotic conditions. In other works a niche was referred to as the "ecological role" of species in a habitat (Elton 1927, MacArthur and Levins 1967) where the focus moved to the effects that organisms have on their environment by, for example, resource consumption. The modern niche concept joins historic definitions, whereby the niche describes organisms as the sum of their ecological requirements that allow them to occupy niche space that meet those requirements. Theoretically, in the case of unlimited niche space, a species can occupy its 'fundamental niche' (Hutchinson 1957) in which every aspect of its ecological requirements can be met. Under natural conditions however, species face restrictions

to available niche space due to competition or other limiting factors; they occupy their 'realized niche' (Hutchinson 1957) in which their growth or reproduction may be limited. According to the concept of the ecological niche, the total niche overlap of two species is followed by competitive exclusion of one of the species (Gause and Witt 1935).

For plant species the range of trophic niches is limited as they generally require the same set of resources; mineral nutrients, water, light and CO2 (Harper 1968), implying that niche overlap is great. One form of niche differentiation in plants is based on their specific resource requirements. Tilman (1977) studied how organisms share nutrient resources in more detail, using planktonic algae as a model system. He grew two species of algae under conditions with two limiting resources and showed empirically that species co-existence is based on a trade-off in the minimum resource levels essential for each species' survival. He found that if two resources were limiting and for each resource one of the species had lower minimum requirements, both species could coexist. He summarised his findings in the so called R* or resource ratio theory where R* defines a set of species-specific resource concentrations that the respective species generates at equilibrium (Tilman 1982).

Soil nutrient mobility and plant competition

Tilman's R* is a widely acknowledged explanation for plant species co-existence but has been the subject of many discussions. The R* theory is also referred to as "concentration reduction theory" because it is based on the assumption that organisms reduce concentrations of a common resource pool up to specific equilibria (Craine et al. 2005). Tested in aquatic systems, Tilman's experiment was carried out in a homogenous environment where the ability of organisms to reduce nutrient concentrations was spatially unrestricted. Planktonic algae in water may therefore affect the surrounding resources differently from plants in soil. Plants generally affect the nutrient resources in close proximity to their root surfaces and rather than depleting the regional resource pool they pre-empt resources locally and create concentration

gradients (Craine et al. 2005). Mineral nutrients are transported to the root surface through the soil by diffusion, mass flow or root interception (Marschner 1986, Casper and Jackson 1997) or they enter plant roots through mycorrhizal hyphae. Dissolved minerals are transported by mass flow, with soil water movement controlled by transpiration, irrigation and evapotranspiration (Marschner 1986). Less soluble minerals are readily adsorbed to soil particles and move mostly by diffusion, following concentration gradients. Nutrient supply by diffusion is only effective over short distances, restricted to the close proximity of root surfaces, and transport is considerably slower than by mass flow (Barber et al. 1963). Huston & DeAngelis (1994) suggested that the mobility of nutrients may affect the ability of plants to affect nutrient concentrations in a resource pool and potentially compete with others. The pre-emption of nutrients by plants should be more effective for mobile nutrients because they can be transported from the resource pool towards the root surface following a concentration gradient.

Nitrogen mobility

Nitrogen is besides water and light the most limited, and therefore contested, resource in grassland ecosystems (Tilman 1987). The two soil nitrogen forms that contribute to plant nutrition, ammonium (NH_4^+) and nitrate (NO_3^-) differ considerably in their transport towards roots. During microbial ammonification NH_4^+ is produced from organically bound nitrogen. During nitrification NH_4^+ is oxidised microbially to nitrite (NO_2^-) and then to NO_3^-. Due to its positive charge NH_4^+ binds to negatively charged soil particles and organic matter. NO_3^- on the other hand is water soluble and transported with soil water. Consequently NO_3^- can reach plant roots by mass flow whereas NH_4^+ is transported via diffusion. High water supply and an excess in NO_3 - N in soils frequently causes nitrogen leaching, and consequently the application of organic fertilizer in agriculture often involves major nitrogen losses and groundwater pollution. Nitrification inhibitors such as DMPP (3,4-dimethyl pyrazole phosphate) are commercially

used in order to avoid oxidation from NH_4^+ - N to NO_3 – N and thereby reduce nitrogen leaching and increase fertilizer efficacy (Zerulla et al. 2001, Yu et al. 2007, Hua et al. 2008, Villar and Guillaumes 2010). In the study presented in **Chapter 1** I use a nitrification inhibitor in order to affect the ratio of mobile to immobile mineral N forms in soils and to test effects of soil nutrient mobility on plant competitive interactions.

Arbuscular mycorrhizal fungi and soil nutrient mobility

The association of plants with soil fungi is one of the oldest between organisms. Arbuscular endomycorrhiza (AMF) can be found in the roots of ~90% of terrestrial plants (Smith and Read 1996). So far only ~200 fungal species belonging to the phylum Glomeromycota (Schüβler et al. 2001) have been identified, which implies little host specificity of the fungi (Smith and Read 1996). The association is mutualistic, meaning that both plant and fungi benefit from the interaction. However, closer analysis has shown that the association can extend from highly beneficial to antagonistic for the plant hosts (Johnson et al. 1997, Kiers and van der Heijden 2006, Wagg et al. 2015), whereas for AMF species the association is obligate; plant host effects are therefore always positive. At the base of the plant-fungi mutualism is an intra-radical hyphae system through which plant and fungi exchange nutrient resources (Hodge et al. 2010). The AMF fungi receive photosynthetic assimilates from their plant host whilst fungi typically benefit plants by the provision of soil nutrients, mainly phosphorus (P) and nitrogen (N), although fungal effects on the plant hosts are variable and have been found to range from increased drought resistance to protection from pathogens (Gange and West 1994, Newsham et al. 1995). Once fungi have colonised roots they grow extra-radical hyphal systems that can expand the plant's rhizosphere by 10-40mm hyphal length per mm root length (Giovannetti et al. 2001), or two orders of magnitude more than the length of colonized roots (Leake et al. 2004) and estimated total hyphal network length of up to 120 cm per root entry point (Friese and Allen 1991). AMF fungi provide plants with P which can make up 80% of

their total P uptake (Smith and Read 1996). Increased P supply is based on the physical extension of the nutrient acquisition area by hyphal growth and fungal enzymatic activity that allows fungi to access P sources otherwise not available to the plant (Allen et al. 2003). The symbiosis with AMF fungi can therefore be essential for the plant host, especially in P limited environments. External hyphae growth generally forms large hyphae systems that connect plants into a common mycorrhizal network (CMN) and due to little host specificity of the fungi, plants of different species can be connected in hyphal networks (Newman 1988, Giovannetti et al. 2006). In this study I assume that the mobility of nutrients between plant rhizospheres is greater due to CMNs and I will disconnect plants from CMNs in order to reduce the nutrient mobility between them.

Space and plant competition

The spatial ecology of plant populations has evolved into a well-considered research field when analyzing competitive interactions and plant community assembly (Horn and MacArthur 1972, Shmida and Ellner 1984, Tilman 1994, Rees et al. 1996, Tilman and Kareiva 1997, Bolker and Pacala 1999, Goldberg et al. 1999, Chesson and Neuhauser 2002, Amarasekare 2003, Milbau et al. 2007).

The mechanisms identified as crucial for species co-existence are species niche heterogeneity, environmental heterogeneity or a trade-off that allows weaker competitors to be superior and persist in other ways or in other locations (Whittaker 1965, Amarasekare 2003, Barot and Gignoux 2004). The sessile lifestyle of plants limits their interactions to the close neighbourhood (Stoll and Weiner 2000, Vogt et al. 2010). Clonal growth (Eriksson 1986) and seed dispersal are the most likely ways for plants to interact with their environment over longer distances.

The consequence of dispersal limitation in plant communities is the frequently observed formation of conspecific aggregates (Rees et al. 1996, Herben et al. 1999). Spatial aggregation

of conspecifics has been proposed to promote co-existence as aggregates create locations in which plants more frequently interact with conspecifics (Murrell et al. 2002, Barot and Gignoux 2004). Weak competitors would therefore benefit as they are protected from interactions with superior species (Weiner and Conte 1981, Rees et al. 1996, Murrell et al. 2001, Stoll and Prati 2001, Monzeglio and Stoll 2005, Mokany et al. 2008, Wassmuth et al. 2008, Porensky et al. 2012). The edges of conspecific clusters are vulnerable to interactions with superior neighbours and eventually better competitors invade the cluster, which is why conspecific aggregates may slow down competitive exclusion but do not promote stable co-existence (Chesson and Neuhauser 2002). It has been debated to which extent spatial patterns in plant communities drive species co-existence (Murrell et al. 2001, Rejmánek 2002).

Co-existence theory suggests that the spatial structure of plant communities can prevent competitive exclusion. Based on the niche theory, space as well as time can be viewed in the same way as trophic resources (Barot and Gignoux 2004). Co-existence based on environmental heterogeneity suggests that patches of varying conditions are occupied by different species, where each species is the best competitor in its patch (Chesson 2000b). Temporal variation in environmental conditions creates niches in a similar manner (Chesson 2000a). Spatial and temporal environmental variation can be created by exogenous factors such as grazing or resource patches, or by endogenous factors as plants shape the environment around them by, for example, shading and nutrient preemption. The species distribution and density in plant communities can therefore determine environmental heterogeneity. In order to promote co-existence by endogenous heterogeneity the specific mechanism has to generate "holes" (Murrell and Law 2003, Barot and Gignoux 2004) that could be occupied by otherwise inferior competitors.

Murrell & Law (2003) proposed a mechanism in which endogenous factors create "holes" in the environment and consequently may promote stable co-existence. They suggest that the intensity of competition between conspecifics is greater over long distances whereas

competition between heterospecifics is more intense at short distances; a mechanism they introduced as “heteromyopia”. Here ultimately, strong intraspecific competition over longer distances will create holes for inferior heterospecifics to occupy. To date Murrell & Law's (2003) theory remains hypothetical and empirical evidence for the existence of “heteromoypia” is scarce. With this study I aim to provide empirical evidence for the differentiation of nutrient competition distances between conspecific and heterospecific neighbours.

Thesis Outlook

In this thesis I explore two major theories that have been proposed to affect plant competitive interactions. In the first two chapters I focus on the theory that supports the effect of soil nutrient mobility on the strength of plant competitive interactions and in the third chapter I am studying spatial aspects of competitive interactions and the concept of heteromyopia. In all chapters my aim is to test the proposed theories empirically and assess their validity for explaining plant competitive interactions and community assembly.

I set up a glasshouse experiment in which I factorially manipulated species diversity and nutrient mobility. The nutrient mobility treatment was based on differences in the mobility properties of the two soil nitrogen forms, ammonium and nitrate. I used a nitrification inhibitor (DMPP) in order to reduce the ratio of mobile nitrate to immobile ammonium in soils and tested whether this has an effect on the biomass production of different plant species and their uptake of locally applied nutrient tracers. The diversity treatment ranging from monocultures to four species mixtures, allowed me to determine whether nutrient mobility effects differed between intraspecific and interspecific plant interactions and detect potential implications for plant community composition (**Chapter 1**).

In a second glasshouse experiment I wanted to test whether the disconnection of plants from a common mycorrhizal network and the resulting reduced nutrient mobility between their

rhizospheres affects their competitive interactions (**Chapter 2**). Here, I used the same factorial design as in Chapter 1.

The spatial structure of plant communities, in particular the spatial aggregation of conspecifics, has been suggested to stabilize species co-existence if interspecific competition is greater over short distances, whereas intraspecific competition is greater over long distances (heteromyopia). I wanted to test whether such a pattern can be found in the nutrient uptake of plant neighbourhoods. I set up a glasshouse experiment and a field study and measured the uptake of nutrient tracers in conspecific and heterospecific neighbour plants at increasing distances from a labelled focal species (**Chapter 3**).

The findings presented in this thesis give insights into, to date, widely untested theories on plant competitive interactions and demonstrate their importance for community structure and species co-existence.

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Chapter 1

Does low soil nutrient mobility reduce competition of neighbour plants? An experimental approach

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Abstract

Aims

Resource competition theory suggests that plants compete by reducing available resource concentrations in their environment. In terrestrial environments, the effect plants can have on resource pools may be restricted locally due to limited nutrient mobility. We aimed to test empirically whether reduced nutrient mobility induced by diffusive limitations affects competitive interactions between plants and whether this effect is propagated through communities of varying species richness.

Methods

We set up a glasshouse experiment and manipulated nutrient mobility and plant species richness in a factorial design. Nitrogen mobility was altered using the commercial nitrification inhibitor DMPP (3,4-dimethyl pyrazole phosphate). In order to track nutrient movements in soils we used isotopic labelling techniques. Competitive effects were quantified by relative isotope uptake, sequential biomass estimation, biomass and nutrient pool measurements from three harvests and were analyzed at individual and community level.

Important findings

We found that the application of DMPP increased the growth and nutrient status of plants. The evenness in DMPP treated pots was reduced for shoot biomass and N pools. We cannot confirm that the decreased ratio of mobile to immobile N forms in the soil affected competitive outcome. Instead we found that increased nutrient supply, which was likely confounded with the treatment, increased plant productivity and belowground competition, and thereby potentially counteracted effects of reduced nutrient mobility on competition.

Keywords

Plant competition, nutrient mobility, DMPP (3,4-dimethyl pyrazole phosphate), diffusion, mass flow

Introduction

Given that plants compete for largely the same set of resources, one of the most fundamental but also least understood question in plant ecology is how species coexist. Aboveground, light seems to be the primary limiting factor (Hautier et al. 2009), whereas soil nutrients, in particular nitrogen (Vitousek and Howarth 1991) are important belowground. Plant species differ in their ability to deplete soil nutrients, and the ones able to draw down available nutrients to very low concentrations may thus be able to displace other species from the community. This idea is at the core of Tilman's resource ratio theory (Tilman 1977) that was originally developed for planktonic algae communities but has also been applied to terrestrial plant communities, with mixed success (Craine et al. 2005).

Whether species can outcompete each other by preempting soil resources depends on the distance over which this competition mechanism operates. If soil nutrient transport is limited, the efficiency of such competition mechanisms may decrease with distance, facilitating co-existence (Huston and DeAngelis 1994). Indeed, modelling studies have shown that nutrient mobility can modulate competition for soil nutrients (Huston and DeAngelis 1994, Raynaud and Leadley 2004, Raynaud et al. 2008). To the best of our knowledge, however, there is only one experimental test of these effects to date (Wilberts et al. 2013).

Competition for belowground resources is inherently difficult to study, because nutrients are transported by a range of biotic and abiotic mechanisms, which are element-specific. In general, the extent to which plants can preempt a resource will depend on the rate at which that resource can be transported to its roots (Raynaud & Leadley 2004; Biondini 2001). In soils, nutrients reach root surfaces by mass flow and diffusion (Marschner 1986). Root interception also is important for plant nutrition (Marschner 1986, Casper and Jackson 1997), but is independent of nutrient mobility and we will therefore not discuss it. Readily soluble minerals are mainly transported by mass flow, with water movement towards roots controlled mainly by

transpiration (Marschner 1986). In contrast, nutrients readily adsorbed to soil particles move mostly by diffusion, following concentration gradients. Nutrient supply by diffusion only is effective over short distances, i.e. in the close proximity of root surfaces, and diffusive transport generally is considerably slower than mass flow (Barber et al. 1963).

Nitrogen is the most growth limiting resource in terrestrial ecosystems (Tilman 1987). Although plants can use organic nitrogen forms, ammonium (NH_4^+) and nitrate (NO_3^-) are the dominant plant nitrogen sources in most ecosystems (Marschner 1986). During ammonification, NH_4^+ is produced by microbial mineralization of organically bound nitrogen. During nitrification, NH_4^+ is oxidized microbially to nitrite and then NO_3^-. NH_4^+readily sorbs to mineral surfaces and is therefore mainly transported by diffusion. In contrast, NO_3^- is readily soluble and often transported by mass flow.

In the present study, we harness the fact that $NH_4^+$is less mobile than NO_3^- to test the hypothesis that low nutrient mobility reduces competition of neighbor plants. We set up a glasshouse experiment in which we manipulated soil NH_4^+ to NO_3^-- ratios using the commercial nitrification inhibitor DMPP (3,4-dimethyl pyrazole phosphate), in addition to applying N fertilizer in NH_4^+ and NO_3^- form. This nutrient mobility treatment was factorially combined with a plant diversity treatment, comprising of communities with 1, 2 and 4 species. Competition was quantified at the individual level by measuring growth curves for each plant. We further determined plant nitrogen (N) and phosphorus (P) contents, and tracked nutrient movement between neighbor plants using N (^{15}N) and P (^{32}P and ^{33}P) isotopes. The factorial diversity treatment enabled us to quantitatively separate intraspecific from interspecific interactions by using the monocultures as reference for the effects within the mixture treatments, and to account for potential species-specific effects of the applied treatment, e.g. preferences for NH_4^+ or NO_3^-. Specifically, we hypothesized that a reduced NO_3^- to NH_4^+ ratio would 1) reduce competition between neighbors; 2) increase evenness (i.e. reduce dominance)

in communities; 3) decrease size inequality between conspecifics and 4) decrease selection effects sensu Loreau and Hector (2001).

Materials and Methods

Experimental Design

We set up a glasshouse experiment in which we factorially manipulated nutrient mobility and plant species richness. In total, the experiment comprised of 224 plant communities that were organized in four replicate blocks. Plant shoots were cut 4 cm above ground after 10 and 18 weeks, and at ground level when the experiment was destructively harvested after 28 weeks. Between harvests, we measured individual plant size traits and estimated their shoot biomass using allometric equations. Nitrogen and phosphorus contents of plants were measured at each harvest, and nutrient movement between pot positions tracked with N and P isotope tracers.

We divided round pots (3 L volume) into quadrants and planted pairs of seedlings of the same species in each quadrant. The rationale of this setup was to increase individual density, thus increasing competition while maintaining a sufficiently large pot size (Poorter et al. 2012). We treated the pair of individuals in one quadrant as unit in all analysis, which can be justified based on the modular character of plant growth and the constant yield law (Weiner and Freckleton 2010). As a result, replicates were not lost in case one of the individuals died. The pots were filled with a nutrient poor grassland soil (see below for details). We added 1 g of shredded, oven-dried ^{15}N-enriched sheep feces to soil of one quadrant and equal amounts of unlabeled sheep feces to the other positions. The feces served as traceable, slowly mineralizing nitrogen source. We periodically added an additional mineral ^{15}N tracer as described below. Aboveground competition between quadrants was minimized by 20 cm tall mesh screens. Belowground, quadrants were not separated allowing unconstrained root competition.

We chose eight temperate grassland species and arranged them in two species pools containing two non-legume forbs (*Plantago lanceolata* and *Prunella vulgaris* or *Plantago media* and *Hierarcium pilosella*) and two grasses (*Festuca pratensis* and *Holcus lanatus* or *Bromus erectus* and *Anthoxanthum odoratum*) respectively. Each pool was divided into two blocks and for each block we planted communities containing 1, 2 or 4 species in all possible compositions. In each block community composition was replicated twice for monocultures, four times for 2-species and 12 times for 4-species-mixtures. Because the distance between pot positions varied and neighbors in opposite positions were growing further away than neighbors beside each other, we arranged species positions differently in replicates of 2-species and 4-species mixtures. We established six control pots for each species in which only one quadrant was planted with two individuals, i.e. there were no competitors in the neighbor quadrants.

Nitrogen mobility treatment and nutrient tracing

We applied the commercial nitrification inhibitor DMPP (3, 4-Dimethylpyrazole/H_3PO_4; K+S Nitrogen GmbH, Mannheim, Germany; 10 mL of 0.4 % DMPP solution per pot) to increase soil NH_4^+ (low mobility treatment) relative to NO_3^- concentrations. In order to correct for phosphorus added with DMPP the remaining pots (high mobility treatment) received 10 ml of 0.03 M KH_2PO_4 solution. DMPP and KH_2PO_4 additions were repeated biweekly. From the first clipping to the end of the experiment, we further added 10 mL of 4 mM $(^{15}NH_4)_2SO_4$ and 8 mM $^{15}NO_3^-$ (Cambridge Isotope Laboratories, Inc., Andover, MA, United States) solution to the labelled quadrants of low and high N mobility pots, respectively; equal amounts of non-labelled KNO_3 and $(NH_4)_2SO_4$ were added to the remaining quadrants. This application was repeated three times at intervals of two weeks. To trace P uptake from neighbor quadrants, six weeks after transplantation and after each harvest 2 mL (0.5 MBq) of either ^{32}P- or ^{33}P-H_3PO_4 solution were added to the ^{15}N labelled quadrant and the other isotope tracer to another quadrant of the same pot.

Plant growth and nutrient uptake

We measured the number of leaves and shoots and the width and length of the three largest leaves of each individual every ten days. Shoot biomass was then estimated using species-specific allometric equations that were obtained by linear regressions of log-transformed harvested shoot biomass vs. log-transformed plant traits at harvest (Table 1).

All biomass samples were dried at 80 °C and weighed. The two individuals of each quadrant were combined, ball-milled in 5 ml Eppendorf tubes, and ^{15}N abundance measured by isotope ratio mass spectrometry (ETH, Eschikon, Switzerland, IRMS type). For cost reasons, all samples were only analyzed for the third (final) harvest. One replicate per pool (1/2 of the pots) was analyzed for the second harvest, and one replicate of the 1st species pool (1/4 of the pots) on the first harvest.

In order to determine total phosphorus (P), ^{32}P and ^{33}P concentrations in plants, fresh leaf subsamples of the individuals of one quadrant were combined and ashed for 3 hours at 600 °C. The ash was dissolved in 2 mL hot 0.1 M sulfuric acid and 5 mL ddH_2O before the solution was filtered (MN 615, Macherey-Nagel GmbH & Co. KG, Düren, Germany). ^{32}P and ^{33}P abundances were determined by liquid scintillation counting (TriCarb, QuantaSmart, Ultima Gold scintillation cocktail, Perkin Elmer, Waltham, MA). P concentrations were measured by automated colorimetry (San^{++}, Skalar Analytical B.V., Breda, Netherlands).

Setup and growing conditions

Prior to experimental set up, seeds (Appels Wilde Samen GmbH, Darmstadt, Germany) of all species were germinated and kept for 8 weeks. Seedlings were cut once before transplantation into experimental pots. The pots were lined with 20 mm drainage mats (Enkadrain, Schöllkopf AG, Rümlang, Switzerland). We used a mixture of 80 % quartz sand (BR Bauhandel AG, Rümlang, Switzerland) and 20 % nutrient-poor soil from an extensively

grazed calcareous grassland in north-western Switzerland. The soil is a Rendzina-type silty clay loam (30 % clay, 56 % silt, 14 % sand) characterized by a 10—15 cm neutral to slightly basic top soil (pH ≈ 7-8) and a calcareous base. The soil was air-dried for three weeks and sieved (4 mm). Temperatures were kept between 13 and 23 °C during the day and between 11 and 20 °C at night. Relative humidity was kept between 40 and 50 % and lights were automatically switched on between 6:00 and 22:00 when light intensity was below 20 klx. Each pot received 150 mL of water three times per week.

Data analysis

We tested for effects of nutrient mobility and species richness on plant shoot biomass, shoot relative growth rate (RGR), and shoot N and P pools at the quadrant and community level using analysis of variance (aov function of R 3.2; http://www.r-project.org). Data was log-transformed to achieve a normal residual distribution. To test for species richness effects we ran the same model again without log transformation. Quadrant-level models contained the terms summarized in Table 2 (Appendix). Within-pot terms were dropped for analyses at the pot level.

Effects of species richness on community shoot biomass and nutrient pools in 4-quadrant communities were partitioned into complementarity and selection effects according to Loreau & Hector (2001). Effects of nutrient mobility on selection and complementarity effects were analyzed using the following model terms which are a subset of model terms summarized in Table 2 (Appendix): (1) Pool, (2) log (species richness), (3) community composition, (4) DMPP, (5) log (species richness) x DMPP.

Differences in shoot biomass and RGR (calculated from shoot biomass estimates) between 1-quadrant controls and 4-quadrant monocultures were determined using analysis of variance with following terms: (1) Pool, (2) block (3) number of individuals, (4) species, (5) number of

plants x species, (6) DMPP, (7) number of plants x DMPP, (8) pot, (9) time of biomass estimate (TOE), (10) TOE x number of plants, (11) TOE x Species, (12) TOE x DMPP, (13) TOE x species x DMPP. For the analysis of effects between times of shoot biomass estimate we subset data to time intervals and dropped TOE from the model.

Results

Competition –free (1-quadrant) controls

Measurements at harvest showed that individuals of all eight species produced significantly more shoot biomass ($F_{1,61}$=1481.6, P=0.001) in Competition-free controls compared to individuals grown in 4-quadrant monocultures (Fig. 1 a, b). The increased individual growth in 1-quadrant communities resulted in a community-level biomass that was only 17% below the one of 4-quadrant monocultures (Fig. 2). The decrease in biomass production due to competition was unaffected by the nutrient mobility treatment (Fig. 1 a, b). DMPP marginally significantly increased shoot biomass in 1-quadrant controls ($F_{1,28}$=4, P=0.06) at first harvest (Fig. 1 a, b, show mean of all harvests).

Shoot relative growth rates (RGR) calculated from biomass estimates (derived from plant trait measurements and allometric relations) showed little difference between 1-quadrant and 4-quadrant monocultures between the first two shoot biomass estimates (Fig. 3). Between the second and third biomass estimate, approximately 10 days later ($F_{1,49}$=15.8, P=0.001) and thereafter, 1-quadrant controls had significantly greater RGRs (Fig. 3). DMPP increased RGRs derived from biomass estimates marginally significantly during the first growth period ($F_{1,28}$=3.6, P=0.07) and significantly during the second growth period ($F_{1,27}$=10.1, P=0.004) (Fig. 3).

Community-level effects

Species richness did neither affect community shoot biomass nor community shoot nitrogen and phosphorus pools (Fig. 2). DMPP increased clipped aboveground biomass at the first ($F_{1,129}$=13.2, P=0.001) but not the subsequent harvests (Fig. 2, shows mean of all harvests). DMPP application did not affect amounts of N removed with the harvested biomass (Fig. 2). We found 36% more P in clipped shoot biomass under DMPP application ($F_{1,129}$=88.6, P=0.001) (Fig. 2), and N to P ratios were significantly lower ($F_{1,129}$=70.4, P=0.001). Total amount of ^{15}N ($F_{1,129}$=25.7, P=0.001) tracer in communities was significantly decreased with low nutrient mobility treatment. Community ^{32}P ($F_{1,129}$=3.8, P=0.06) tracer amount tended to increase with DMPP (data not shown).

Individual-level effects

Species richness generally had no effect on individual shoot biomass, although some species tended to increase shoot biomass with community species richness whereas others had reduced shoot biomass by trend (Fig. 1 a, b). When species were analyzed separately, no effects were found except for a decrease in harvested shoot biomass in *F. pratensis* ($F_{1,3}$=78.3, P=0.004,) and an increase with species richness in *P. lanceolata* ($F_{1,3}$=13.6, P=0.04) (Fig. 1 a). Species richness had no effect on individual N and P pools and N to P ratios (Fig. 1 a, b).

DMPP increased the average individual shoot biomass by 8% at the first harvest ($F_{1,129}$=5.4, P=0.02) (Fig. 1 a, b) but had no effect at subsequent harvests. Individual N pools were unaffected by DMPP but P pools were significantly greater ($F_{1,129}$=118, P=0.001) and N to P ratios ($F_{1,129}$=77.1, P=0.001) reduced with DMPP across harvests (Fig. 1 a, b). DMPP effects on individual shoot biomass ($F_{12,129}$=1.9, P=0.04, DMPP x composition) and N pools differed with community composition ($F_{12,43}$=2.2, P=0.03, DMPP x composition) at second harvest. N pools at first harvest showed marginally significant interactions with species richness

($F_{1,5}$=5.1, P=0.07, DMPP x species richness) with greater increases with DMPP in 2-species and 4-species mixtures compared with monocultures. DMPP affected N pools ($F_{6,203}$=2.3, P=0.04, DMPP x species) and P pools species-specifically ($F_{6,203}$=2.33, P=0.034, , DMPP x species) with significant N pool increases in *P.lanceolata* ($F_{1,41}$=5.3, P=0.03) and general P pool increases with exception of *H.pilosella* (Fig. 1 a, b).

Species interactions

To determine the movement of nutrient tracers we analyzed their amount in labelled plants from quadrants of tracer application in relation to tracer in the whole community. Relative ^{32}P amount (arc-sinus transformed) in labelled plants was significantly reduced in species-rich communities ($F_{1,19}$=4.5, P=0.05) (Fig. 6) and for both ^{32}P ($F_{1,126}$=78.1, P<0.001) and ^{33}P tracer ($F_{1,126}$=61.5, P<0.001) we found significantly more ^{15}N and P tracer away from label position at later harvests regardless of the DMPP application (Fig. 6, Fig. 7). DMPP did not affect the relative amount of P or N (Fig. 6, Fig. 7) tracer in label position.

DMPP did neither change selection nor complementarity effects in 4-quadrant communities based on biomass measurements and community N pools across harvests (data not shown). Generally, complementarity effects were positive for shoot biomass and negative for N pools. Selection effects for shoot biomass became insignificantly negative with the nutrient mobility treatment and were negative for N pools regardless of the nutrient mobility treatment. Selection effects for community P pools were negative and significantly smaller ($F_{1,13}$=6.7, P=0.02) with DMPP whereas complementarity effects were negative but remained unchanged.

We determined DMPP effects on evenness based on 1/D evenness index (Simpson diversity index and Hill series) community shoot biomass, N- and P pools. We found that evenness of shoot biomass (arc-sinus transformed) in 4-species-mixtures decreased

significantly with DMPP across all harvests and both species pools ($F_{1,42}$=4.8, P=0.04) (Fig. 4). In 2-species-mixtures shoot biomass evenness was unaffected by DMPP. Evenness of N distribution (arc-sinus transformed) in 4-species communities was significantly reduced with DMPP ($F_{1,42}$=12.2, P=0.002,) but unaffected in 2-species mixtures (Fig. 4). P distribution (arc-sinus transformed) tended to be less even with DMPP in both, 2-species and 4 species- mixtures ($F_{1,113}$=3.4, P=0.07) (Fig. 4).

Intraspecific interactions

Effects of DMPP on intraspecific competition were determined with size inequality between quadrants in monoculture pots and conspecifics in 2–species mixtures using standard deviation of shoot biomass (log), N (log) and P pools (log). We found that DMPP significantly increased standard deviation of shoot biomass between monoculture quadrants and between quadrants of conspecifics in 2-species mixtures ($F_{1,85}$=6.63, P=0.012) (Fig. 5). DMPP tended to increase standard deviation in N pools ($F_{1,85}$=3.62, P=0.061) but did not affect standard deviation of P pools (Fig. 5).

Discussion

Nutrient mobility was suggested to promote competitive interactions between plants (Huston and DeAngelis 1994) but our results do not confirm this hypothesis. We applied a nitrification inhibitor in order to reduce the ratio of mobile to immobile N forms in the soil. The application of the nitrification inhibitor had substantial effects on plant growth and nutrient pools in 4-quadrant communities. Generally plants increased their growth and nutrient status in particular for phosphorus.

Nitrification inhibitors are commonly used in agriculture to enhance the efficacy of organic and mineral fertilizers and increase yield by avoiding direct mineralization of NH_4^+ to more mobile nitrogen forms (NO_3^-) that are potentially lost by leaching (Zerulla et al. 2001, Yu

et al. 2007, Hua et al. 2008, Villar and Guillaumes 2010). For this experiment, we have to consider that the ratio of mobile to immobile N forms was not solely affected, but that the total available N was higher when soils were treated with DMPP due to avoided nitrate losses through leaching and denitrification (Patra et al. 2006). However, we could not find evidence for increased N pools at community level. Effects of DMPP on nitrogen pools at the individual plant level were species-specific, indicating that some species benefitted from the treatment, especially under interspecific competition as indicated by greater N pool effects in more species rich communities. P pools were always positively affected but also showed species-specific differences. DMPP contains P which we corrected for with KH_2PO_4 addition to control pots. Nevertheless, we cannot rule out that P derived from DMPP was more accessible to plants and the increased supply of P caused the positive effects of DMPP on plant growth. We found that the evenness for shoot biomass, N pools, and P pools was reduced, another indication that some species benefitted more from increased nutrient supply and hence became more abundant than others in the community.

Huston & DeAngelis (1994) argued that Tilman's (1977) resource ratio hypothesis would hold only in environments where nutrient supply is low and nutrient mobility is high. They proposed that a good competitor could preempt the nutrients in close proximity to its roots, and consequently create a concentration gradient which will draw more nutrients into the rhizosphere when nutrient mobility is high. So far, there was one attempt to test the effects of nutrient mobility on competition (Wilberts et al. 2013). Besides the effects of nutrient mobility, Wilberts et al. tested whether increased nutrient supply reduced competitive interactions as stated by Huston & DeAngelis (1994). They found that nutrient mobility increased competition of larger plants over smaller plants and therefore supported Huston & DeAngelis' theory. In contrast to the hypothesis that low nutrient supply increases competition, they found that nutrient supply increased competition. These results are in line with our findings that showed increased competition at greater nutrient supply. We could not find that nutrient mobility had

an effect on competition. However, the nutrient mobility treatment we used, was most likely confounded by nutrient supply. Our results suggest that increased nutrient supply counteracted potential alleviating effects of reduced nutrient mobility.

Before Huston & DeAngelis stated their theory on the effects of nutrient supply on competition, it was debated whether soil fertility can affect competitive interactions. There were two views that dominated and motivated the debate. Grime et al. (1987) stated that low productivity or more stressful environmental conditions will allow otherwise less successful "stress-tolerators" to persist over otherwise "good competitors". Hence, competition is less important under low nutrient supply. On the other hand, Tilman (1977) argued that competition can be equally important under any condition because it is based on the intrinsic nutrient requirement trade-offs between species or individuals. Although consensus around their views was small, both theories state that at low productivity selection will be in favor of plants that tolerate low supply rates (Grace 1991). Our findings, in conjunction with previous findings (Wilberts et al. 2013), support Grime's view that high nutrient supply increases the success of "good competitors".

Increased aboveground competition due to enhanced plant growth explains increased competition at high productivity which was supported by empirical studies (Wilson and Tilman 1991, 1993, 1995, Putz and Canham 1992, Hautier et al. 2009). Here, we prevented aboveground competition, but cannot rule out that higher nutrient uptake, resulting in increased nutrient gradients in the rhizosphere of larger plants, leaded to greater competition belowground and increased negative effects on smaller neighbors (Schwinning and Weiner 1998). Decreased evenness for both, shoot biomass and nutrient contents with DMPP in mixtures and increased standard deviation from mean size in conspecifics supports this assumption. Initial size differences between species may have been enhanced with belowground competition. Wilberts et al. (2013) obtained comparable results with a similar experimental setup to our experiment,

argued that increased productivity not solely increased competition aboveground but also competition belowground. We believe our results contribute to increasing evidence that belowground competition at higher productivity may be just as important as aboveground competition.

Here, in order to detect the effects of nutrient mobility on plant competitive interactions we measured plant growth and nutrient uptake of plants, as well as, nutrient movement between them. For this experimental setup under highly controlled conditions, we can conclude that nutrient mobility was not important for plant competitive interactions, but was potentially masked by a more important increase of nutrient supply.

Theoretical modelling approaches, analyzing driving forces of competitive interactions are powerful tools, especially here, where experimental manipulation of soil nutrient mobility is challenging. However, as long as we are lacking empirical evidence, such models remain speculative and sometimes may not even remotely describe natural plant interactions. Our results point in opposite directions of the study by Wilberts et al. (2013) in regards to effects of nutrient mobility on competitive interactions. The commonality of results regarding the importance of nutrient supply on belowground competition underlines the need for further experimental studies on competitive interactions in response to soil nutrient mobility with consideration of nutrient supply rates.

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Table 1: Allometric equations for eight study species. Allometric coefficients derive from log linear regressions of biomass at harvest and plant trait measurements.

Species	Allometric equation (x= shoot biomass estimate)
Plantago lanceolata	-8.085+1.5173*log(leaf number)+1.075*log(leaf length)+0.783*log(leaf width)
Prunella grandiflora	-7.404+1.423*log(leaf number)+1.386*log(leaf length)
Hierarcium pilosella	-4.99+1.09*log(leaf number)+0.599* log(leaf width)
Plantago media	-6.045+0.125*log(leaf number)+0.876*log(leaf length)+2.591*log(leaf width)
Holcus lanatus	-7.394+1.11*log(leaf number)+0.97*log(leaf length)-0.254*log(leaf width)
Festuca pratensis	-7.38+1.276*log(leaf number)+0.639*log(leaf length)-0.802*log(leaf width)
Anthoxanthum odoratum	-6.610+1.246*log(leaf number)+0.572*log(leaf length)+0.673*log(leaf width)
Bromus erectus	-7.506+1.237*log(leaf number)+0.953*log(leaf length)+0.215*log(leaf width)

Figure 1 a, b: Mean (of three harvests) shoot biomass (2 individuals), N pools and P pools of species in a) species pool 1 and b) species in pool 2 when grown without competition (1 quadrant), in monocultures, 2-species or 4-species mixtures. *Festuca pratensis* (F.p), *Holcus lanatus* (H.l), *Plantago lanceolata* (P.l) *Prunella grandiflora* (P.g), *Anthoxanthum odoratum* (A.o), *Bromus erectus* (B.e), *Plantago media* (P.m), *Hierarcium pilosella* (H.P). White bars and symbols represent biomass in control pots. Grey bars and symbols represent biomass in DMPP treated pots. Error bars show standard errors of the mean.

Figure 2: Mean (of three harvests) community shoot biomass, nitrogen and phosphorus pools of 1-quadrant controls, monocultures, 2-species and 4-species mixtures. White bars represent biomass in control pots. Grey bars represent biomass in DMPP treated pots. Error bars show standard errors of the mean. * indicates a significant DMPP effect ($P<0.05$).

Figure 3: Mean estimated biomass of plants (2 individuals) in species pool 1 and species pool 2 when grown without competition, with intraspecific competition (in monocultures) and with interspecific competition (in 2-species and 4-species mixtures) over time (DOY: Day of Year since 1st January 2012). Data derives from first (185-258 DOY) and second growing period (163-327 DOY). Biomass estimates derive from plant trait measurements at five observations in each growing period.

Figure 4: Mean evenness ($E_{1/D}$) (of three harvests) in shoot biomass, N pools and P pools in 2-species and 4-species mixtures when grown in control (white) or DMPP treated (grey) pots. Error bars show standard errors of the mean. * indicates a significant DMPP effect ($P<0.05$).

Figure 5: Mean standard deviation (of three harvests) of shoot biomass, P pools and N pools in monocultures and conspecifics of 2-species mixtures when grown in control (white) or DMPP treated (grey) pots. Error bars show standard errors of the mean. * indicates a significant DMPP effect ($P<0.05$).

Figure 6: Mean fraction of ^{15}N tracer in individuals, growing in location of isotope application of monocultures, 2-species or 4-species mixtures at each harvest. Fractions are based on tracer amount in labelled individuals over tracer in the community. White bars represent individuals in control pots and grey bars individuals in DMPP treated pots. Error bars show standard errors of the mean.

Figure 7: Mean fraction of ^{32}P and ^{33}P tracer in individuals, growing in location of isotope application of monocultures, 2-species or 4-species mixtures at each harvest. Fractions are based on tracer amount in labelled individuals over tracer amount in the community. White bars represent individuals in control pots and grey bars individuals in DMPP treated pots. Error bars show standard errors of the mean.

Figure 1 a

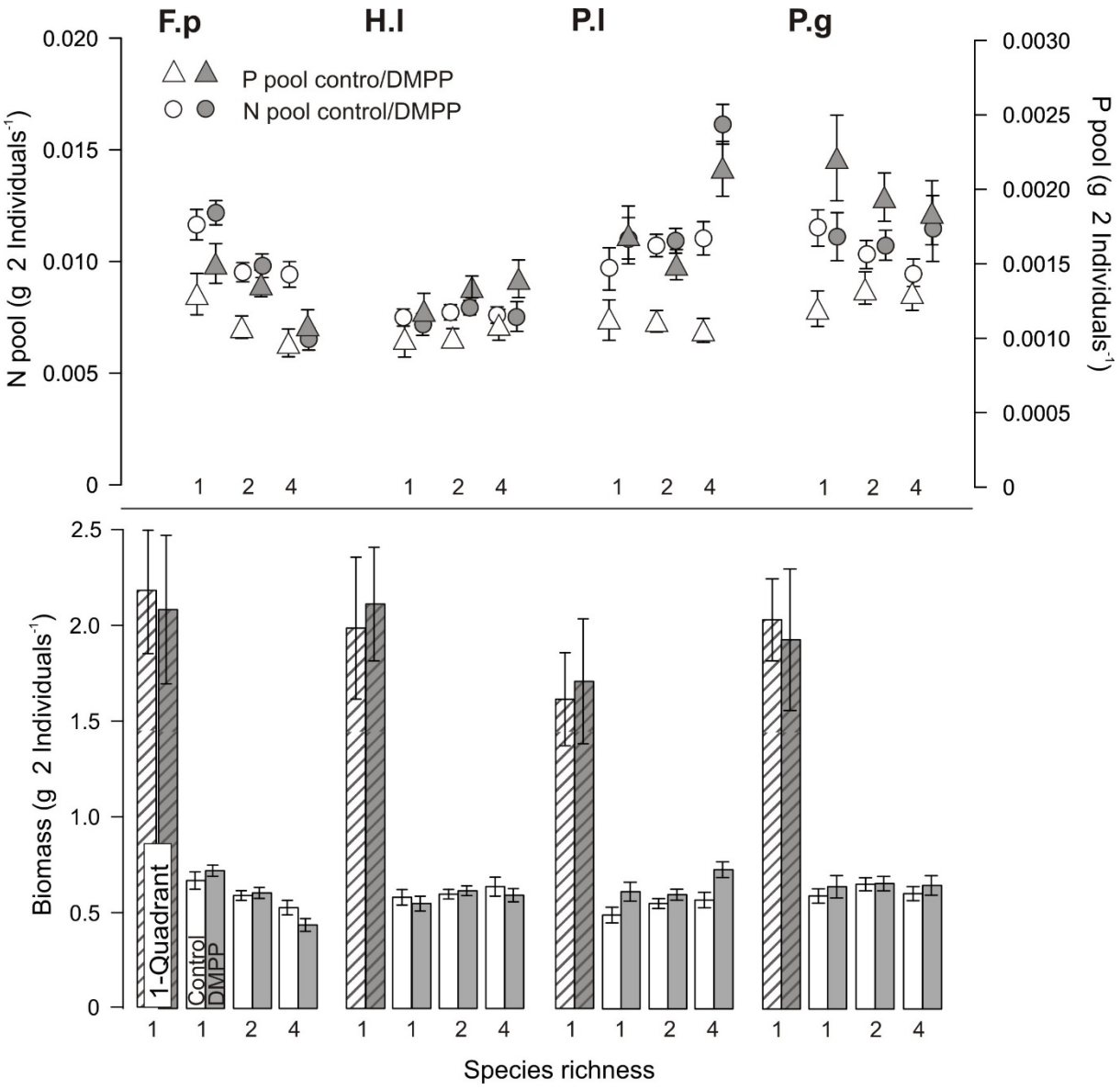

F.p
H.l
P.l
P.g
P pool contro/DMPP
N pool control/DMPP
N pool (g 2 Individuals-1)
P pool (g 2 Individuals-1)
0.020
0.015
0.010
0.005
0
0.0030
0.0025
0.0020
0.0015
0.0010
0.0005
0
1 2 4 1 2 4 1 2 4 1 2 4
Biomass (g 2 Individuals-1)
2.5
2.0
1.5
1.0
0.5
0
1-Quadrant
Control
DMPP
1 1 2 4 1 1 2 4 1 1 2 4 1 1 2 4
Species richness

Figure 1 b

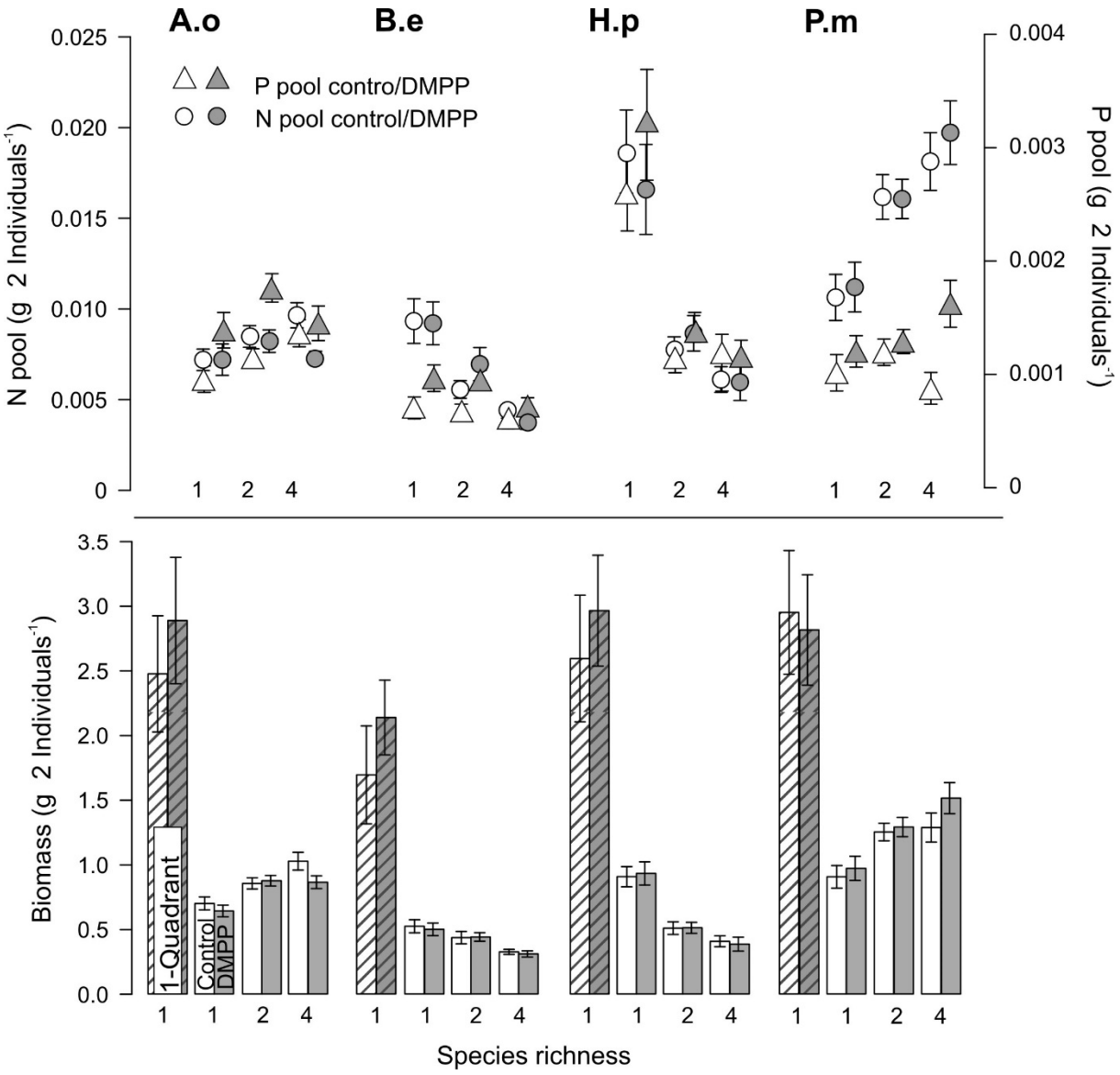

A.o
B.e
H.p
P.m
P pool contro/DMPP
N pool control/DMPP
N pool (g 2 Individuals-1)
P pool (g 2 Individuals-1)
0.025
0.020
0.015
0.010
0.005
0
0.004
0.003
0.002
0.001
0
1 2 4
Biomass (g 2 Individuals-1)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
1-Quadrant
Control
DMPP
1 1 2 4 1 1 2 4 1 1 2 4 1 1 2 4
Species richness

Figure 2

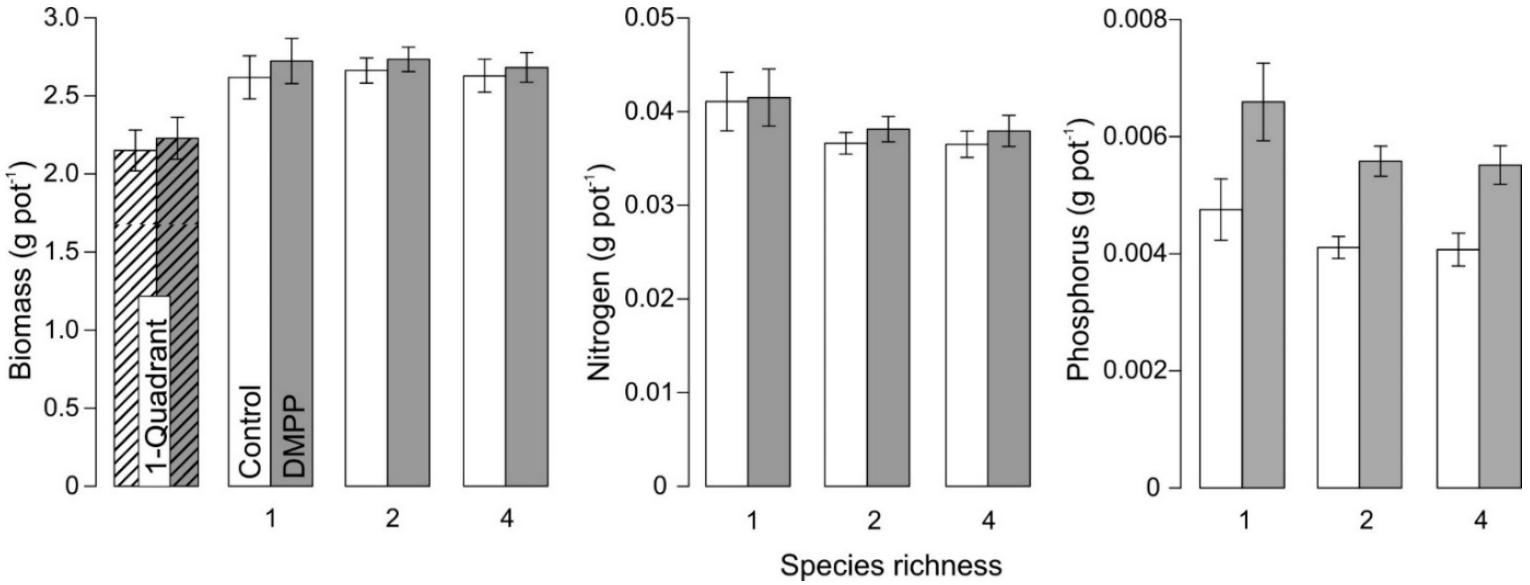

Biomass (g pot-1)
3.0
2.5
2.0
1.5
1.0
0.5
0
1-Quadrant
Control
DMPP
1
2
4
Nitrogen (g pot-1)
0.05
0.04
0.03
0.02
0.01
0
1
2
4
Phosphorus (g pot-1)
0.008
0.006
0.004
0.002
0
1
2
4
Species richness

Figure 3

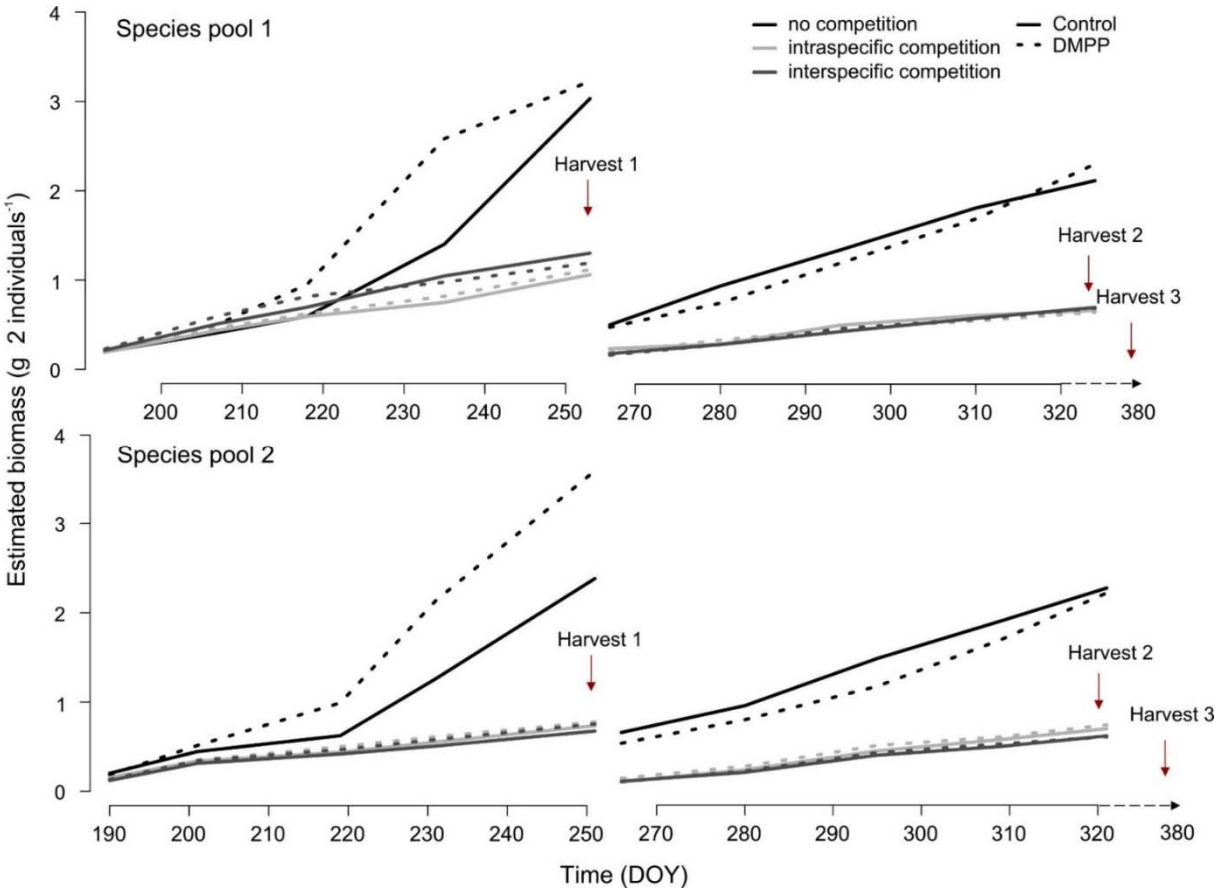

Species pool 1
Species pool 2
no competition
intraspecific competition
interspecific competition
Control
DMPP
Harvest 1
Harvest 2
Harvest 3
Estimated biomass (g 2 individuals-1)
Time (DOY)
0
1
2
3
4
190
200
210
220
230
240
250
270
280
290
300
310
320
380

Figure 4

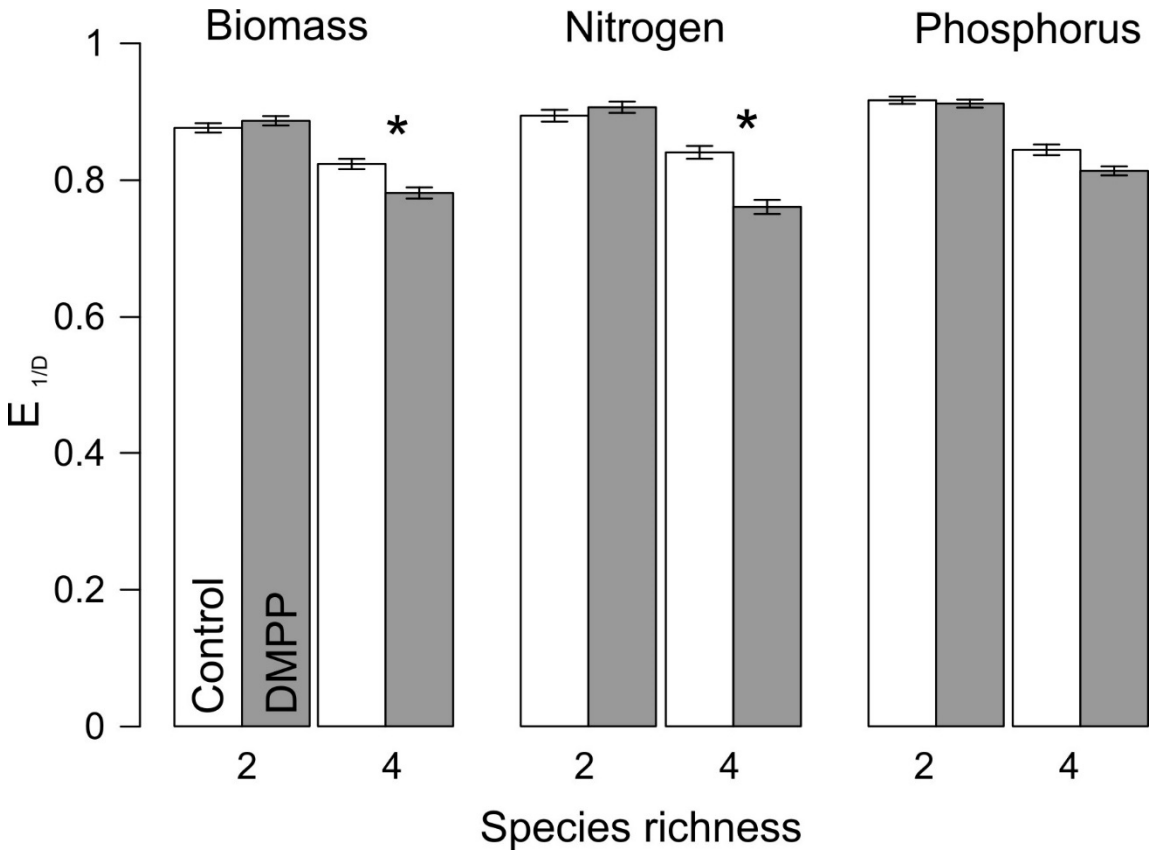

Biomass
Nitrogen
Phosphorus
1
0.8
0.6
0.4
0.2
0
E 1/D
Control
DMPP
*
*
2
4
2
4
2
4
Species richness

Figure 5

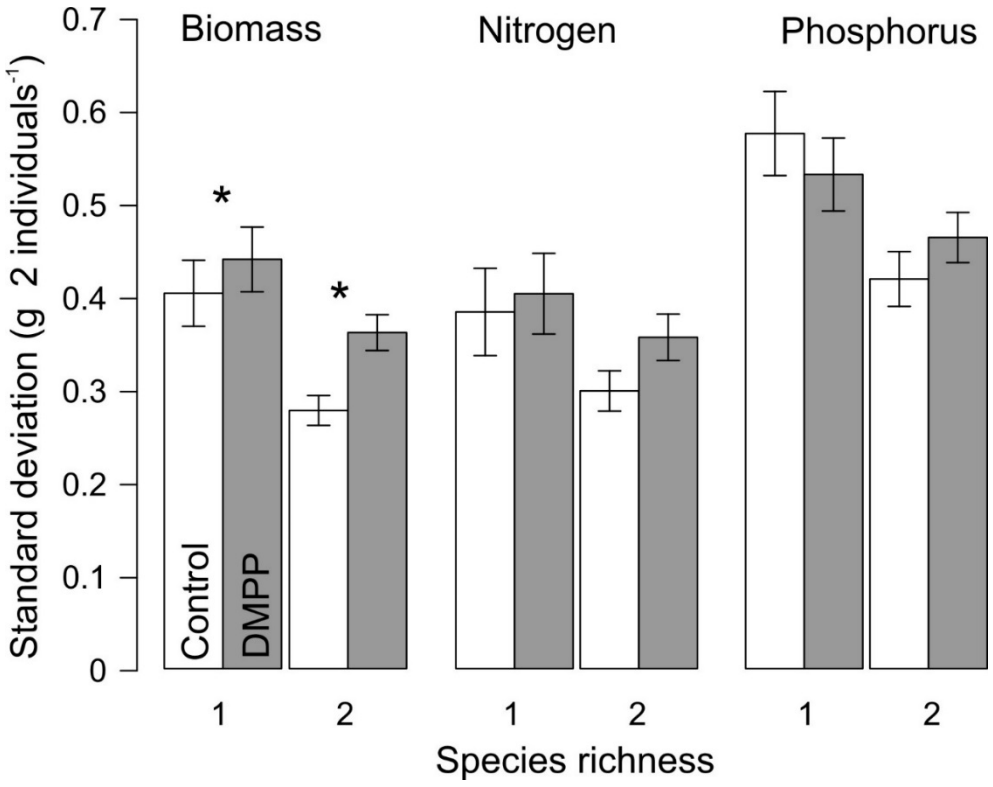

Biomass
Nitrogen
Phosphorus
Standard deviation (g 2 individuals^{-1})
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Control
DMPP
*
*
1
2
1
2
1
2
Species richness

Figure 6

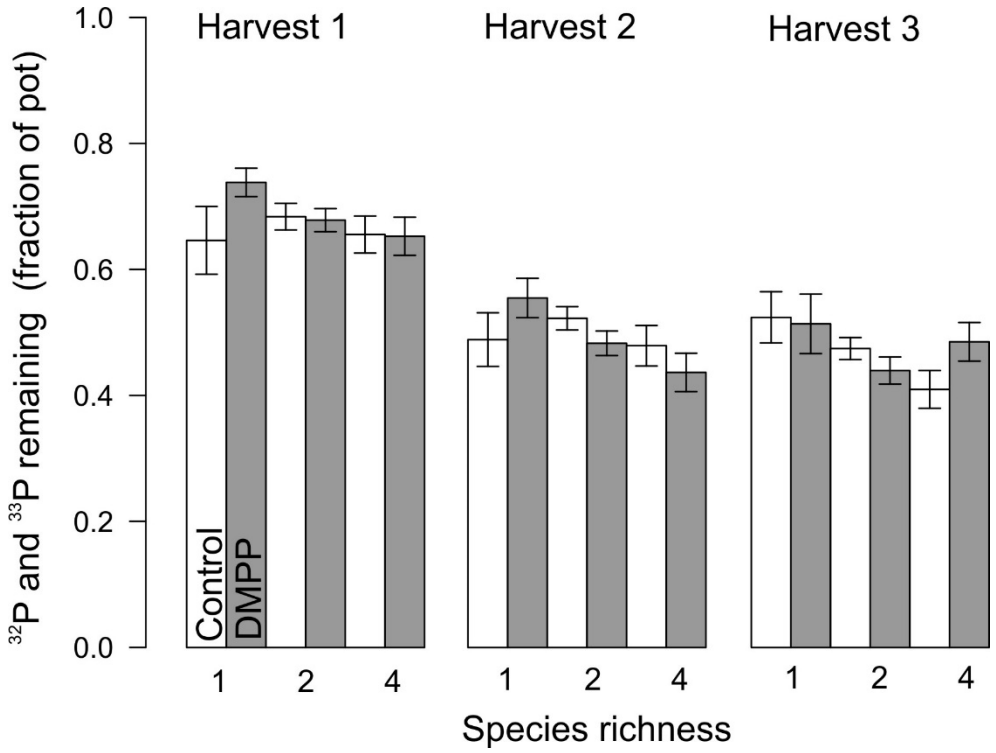
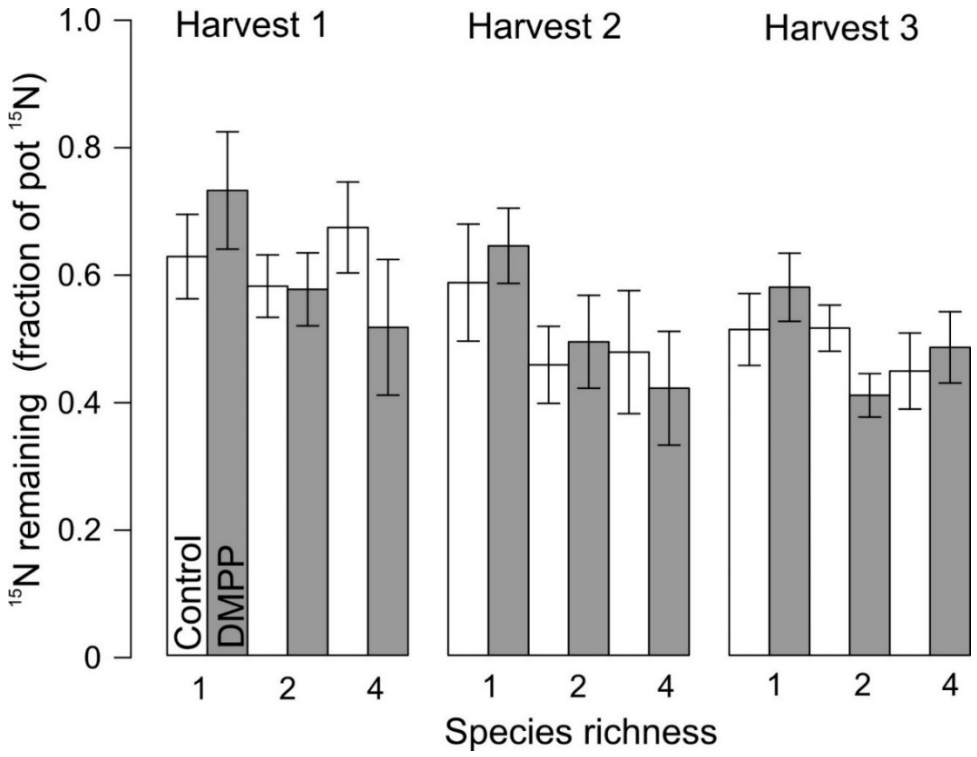

Harvest 1
Harvest 2
Harvest 3
1.0
0.8
0.6
0.4
0.2
0.0
32P and 33P remaining (fraction of pot)
Control
DMPP
1
2
4
1
2
4
1
2
4
Species richness

Figure 7


Harvest 1
Harvest 2
Harvest 3
1.0
0.8
0.6
0.4
0.2
0
^{15}N remaining (fraction of pot ^{15}N)
Control
DMPP
1
2
4
Species richness

Appendix

Table 2*:* Structure and results of analysis of variance

					Individual level				Community level											
Level	Term	df	Error	ddf	Biomass (log)	N (log)	P (log)	N:P ratio (log)	Biomass (log)	N (log)	P (log)	N:P ratio (log)	E 1/D (asin) Biomass	E 1/D (asin) N	E 1/D (asin) P	SD Biomass (log)	SD N (log)	SD P (log)	N15 label pos. (asin)	P32 label pos. (asin)	P33 label pos. (asin)
Block																				
	Pool	1	Block	2	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	**	**	***	n.s	n.s	n.s	n.s	*	n.s
	Residual (Block)	2																		
Composition																				
Species richness (SR)																				
	log SR	1	mixture composition	11	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	**	**	***	n.s	n.s	(*)	n.s	*	n.s
	monoculture vs. mixture	1	mixture composition	11	n.s	n.s	n.s	n.s	—	—	—	—	—		—	—	—	—	—	—	—
Residual																				
	monoculture species	7	mixture composition	11	(*)	*	*	(*)	—	—	—	—	—		—	(*)	**	n.s	***1	*1	***1
	mixture composition	11																		
Pot																				
	DMPP	1	Residual (Pot)	129	n.s	n.s	***	***	*	n.s	***	***	n.s	**	(*)	*	(*)	n.s	n.s	n.s	n.s
Composition x DMPP																				
	DMPP x Species richness																			
	DMPP x log SR	1	DMPP x mixture composition	129	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	**	**	n.s	—	—	—	n.s	n.s	n.s
	DMPP x monoculture vs. mixture	1	DMPP x mixture composition	129	n.s	n.s	n.s	n.s	—	—	—	—	—	—	—	n.s	n.s	n.s	—	—	—
	DMPP x residual composition																			
	DMPP x monoculture species	7	DMPP x mixture composition	129	n.s	n.s	n.s	n.s	—	—	—	—	—	—	—	(*)	n.s	n.s	n.s	n.s	n.s
	DMPP x mixture composition	12			n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	(*)	n.s	n.s
Residual (Pot)		129																		
Population (Species x Pot, mixtures only)																				
	Species	6	Residual (Population)	203	***	***	***	***	—	—	—	—	—	—	—	n.s	n.s	(*)	—	—	—
Species x Composition																				
	Species x log SR	6	Residual (Species x Composition)	6	n.s	n.s	n.s	n.s	—	—	—	—	—	—	—	—	—	—	—	—	—
	Residual (Species x mixture composition)	6																		
Species x Composition x DMPP																				
	Species x DMPP	6	Residual (Population)	203	n.s	*	*	n.s	—	—	—	—	—	—	—	n.s	n.s	n.s	—	—	—
	Species x DMPP x log SR	6	Residual (Species x Composition x DMPP)	6	n.s	n.s	n.s	n.s	—	—	—	—	—	—	—	—	—	—	—	—	—
	Residual (Species x mixture composition x DMPP)	6																		
Residual (Population)		203																		

Harvest x Population																			
Harvest																			
Harvest x Pool		Harvest x Block																	
Harvest x Block																			
Harvest x log SR	2	Harvest x mixture composition	24	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
Harvest x monoculture vs. mixture	2	Harvest x mixture composition	24	n.s	n.s	n.s	n.s	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Harvest x monoculture species	14	Harvest x mixture composition	24	n.s	n.s	n.s	n.s	—	—	—	—	—	—	—	n.s	n.s	n.s	*1	**1	***1
Harvest x mixture composition	24																			
Harvest x DMPP	2	Harvest x Pot	262	**	n.s	n.s	n.s	**	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	(*)
Harvest x DMPP x log SR	2	Harvest x DMPP x mixture composition	24	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	—	—	—	n.s	n.s	(*)
Harvest x DMPP x monoculture vs. mixture	2	Harvest x DMPP x mixture composition	24	n.s	n.s	n.s	n.s	—	—	—	—	—	—	—	n.s	n.s	n.s	—	—	—
Harvest x DMPP x monoculture species	14	Harvest x DMPP x mixture composition	24	n.s	n.s	n.s	n.s	—	—	—	—	—	—	—	(*)	n.s	n.s	n.s	n.s	n.s
Harvest x DMPP x mixture composition	24																			
Harvest x Pot	262																			
Harvest x Species	12	Harvest x Species x Pot	406	***	***	***	***	—	—	—	—	—	—	—	**	*	n.s	—	—	—
Harvest x Species x log SR	12	Harvest x Species x composition	12	n.s	n.s	n.s	n.s	—	—	—	—	—	—	—	—	—	—	—	—	—
Harvest x Species x composition	12																			
Harvest x Species x DMPP	12	Harvest x Species x Pot	406	n.s	n.s	n.s	*	—	—	—	—	—	—	—	n.s	n.s	n.s	—	—	—
Harvest x Species x DMPP x log SR	12	Harvest x Species x DMPP x composition	12	n.s	n.s	n.s	n.s	—	—	—	—	—	—	—	—	—	—	—	—	—
Harvest x Species x composition x DMPP	12																			
Harvest x Species x Pot	406																			

Degrees of freedom derive from the model including all observations from four blocks and three harvests when analyzed for effects on shoot biomass of species at quadrant level (up to 4 observations per pot). When we analyzed for effects on other variables or effects at community level model terms were reduced and degrees of freedom varied. E 1/D= Evenness index for 2-species and 4-species mixtures, SD= standard deviation in conspecifics of monocultures and 2 species mixtures, ^{15}N, ^{32}P, ^{33}P label pos. = nutrient tracer amount in labelled plants (2 individuals) as fraction of total tracer amount in all plants of the pot.[1] here monoculture species refers to species in monocultures and mixtures.

Chapter 2

Disconnection from a common mycorrhizal network reduces complementarity not competition between plants

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Abstract

Aims

The extent to which plants can reduce nutrient pools in soil and thereby potentially compete with others was proposed to increase with nutrient mobility. Arbuscular mycorrhizal fungi (AMF) can increase the nutrient mobility between plants through external hyphae growth. In this study we aim to reveal whether decreased nutrient mobility between neighbour plants induced by the prevention of inter-rhizosphere hyphal growth can reduce competitive interactions and consequently affect community structure.

Methods

We used custom-build pots that allowed us to prevent the development of common mycorrhizal networks (CMN) between neighbouring rhizospheres. We applied the CMN treatment to competition-free controls, monocultures, 2-species and 4-species mixtures and analyzed changes in individual growth, community evenness, size inequality and selection and complementarity effects based on shoot and root biomass and shoot phosphorus pools. We used ^{32}P and ^{33}P tracer to track P nutrient movement between plants.

Important findings

The disconnection from CMNs reduced shoot and increased root biomass in plants. Species complementarity in shoot biomass decreased with the CMN treatment. These results suggest that competition between neighbouring plants was unaffected by lower nutrient mobility from CMN disconnection. Instead, positive effects of CMN connection on plant growth and species complementarity were reduced, potentially outweighing effects of nutrient mobility on competition.

Keywords

Plant competition, Arbuscular mycorrhizal fungi (AMF), Common mycorrhizal network, CMN, Nutrient mobility, Complementarity

Introduction

Competition is the key mechanism behind plant species co-existence (Gause 1934), yet processes that prevent competitive exclusion and promote species diversity are poorly understood. Resource niche partitioning has long been recognized as the mechanism behind plant species coexistence and can explain why more diverse assemblages of species are more productive (Hutchinson 1957, Hector and Hooper 2002, Chase and Leibold 2003, Silvertown 2004). Individuals of different species (heterospecifics) are more likely to require a different resource niche than those of the same species (conspecifics). Thus, a greater species diversity within a community should diversify the resource niche requirements among community members and improve the coexistence and complementarity among individuals (Mayfield and Levine 2010, Turnbull et al. 2012). As most plant species compete to a great extent for the same set of resources, diversity in resource niches appear constrained, yet Tilman (1977) explained niche differentiation as a consequence of trade-offs in species specific minimum requirements. According to Tilman's resource ratio theory, plants can reduce concentrations in a resource pool and thereby displace other species from the community. This theory explained competitive outcomes in homogenous environments with high resource supply. The precondition of classical resource competition theory, that organisms can reduce the concentration of resource pools may be hampered in terrestrial ecosystems with low resource mobility (Huston and DeAngelis 1994). Previous studies demonstrated that nutrient mobility is an important driver of plant competitive interactions in theoretical models (Raynaud and Leadley 2004, Raynaud et al. 2008) and recently published results support the theory empirically (Wilberts et al. 2013).

Nutrient mobility between plants can be promoted through arbuscular mycorrhizal fungi (AMF) hyphae. The mutualism between plants and arbuscular mycorrhizal fungi (AMF) can expand the nutrient acquisition area of plants dramatically (Sanders and Tinker 1971, Rhodes and Gerdemann 1975, Giovannetti et al. 2001) and, depending on availability, (Allen et al.

2003) the amount of phosphorus (P) a plant receives from its mutualistic partner can provide up to 80% of its total consumption (Smith and Read 1996) and consequently improve its performance (Jakobsen et al. 1992, Smith and Read 1996, Smith et al. 2003). AMF can colonise and connect roots of different individuals and because their host specificity is low (Smith and Read 1996), different species can be connected within a common mycorrhizal network (CMN) (Newman 1988, Giovannetti et al. 2006). The presence of CMN was found to have major implications for nutrient allocation (Simard and Perry 1997, Wilson et al. 2006) and competitive outcome between individuals, and consequently are important for structuring plant communities (Read 1997, van der Heijden et al. 1998). In this study we harness the fact that nutrient mobility is greater in plant communities connected in CMNs in order to test whether nutrient mobility affects plant-plant competition.

We set up a glasshouse experiment with 224 specifically designed pots that enabled us to disconnect plants from a common mycorrhizal network while still allowing each plant to be colonised by AMF. The CMN treatment was factorially combined with a plant diversity treatment, consisting of communities with 1, 2 and 4 species. In addition we grew competition-free controls. The factorial diversity treatment allowed us to quantitatively separate intraspecific from interspecific interactions, by using monocultures as reference for effects found within the mixture treatments, along with detecting potential species-specific dependencies on CMN connectedness. We measured competition as individual response in relative growth and biomass production, shoot phosphorus (P) pools, and by tracking nutrient movement between plants using ^{32}P and ^{33}P radio-isotopes.

We hypothesized that the prevention of inter-rhizosphere hyphal growth and AMF network formation would reduce their competitive interactions, resulting in: 1) increased biomass production and nutrient uptake in weaker competitors when CMN are absent. 2)

Increased evenness in biomass and nutrient pools in mixtures, and reduced inequalities in monocultures and 3) reduced selection effects (Loreau and Hector 2001) in mixtures (Fig. 1).

Materials and Methods

Experimental design

We set up a glasshouse experiment in which we factorially manipulated nutrient mobility and plant species richness. In total, the experiment comprised 224 plant communities that were organized in four replicate blocks. Plant shoots were cut to 4 cm after 12 and 25 weeks. After 40 weeks we harvested shoots to ground level and extracted and washed their roots. Between harvests, we measured individual plant size traits and estimated their shoot biomass using allometric equations. Phosphorus contents of plants were measured at last harvest, and nutrient movement between pot positions was tracked using P tracers.

We custom-built squared pots with four compartments (Fig. 2) and planted each quadrant with two seedlings of the same species. The rationale of this setup was to increase individual density, thus increasing competition while maintaining a sufficiently large pot size (Poorter et al. 2012). We treated the pair of individuals as a unit in all analysis, which can be justified based on the modular character of plant growth and the constant yield law (Weiner and Freckleton 2010). As a result, replicates were not lost in case one of the individuals died. The pots were filled with a mixture of quartz sand and nutrient poor grassland soil (see below for details). A wire system in the pot allowed us to cut the hyphal connections between quadrants repeatedly and thereby prevent the formation of CMNs. Above-ground quadrants were divided by 20 cm tall mesh screens in order to avoid competition for light.

Eight temperate grassland species were chosen and assembled into two species pools. Each pool contained two non-legume forbs (*Plantago lanceolata* and *Prunella vulgaris* or *Plantago media* and *Hierarcium pilosella*) and two grasses (*Festuca pratensis* and *Holcus*

lanatus or *Bromus erectus* and *Anthoxanthum odoratum*) respectively. Each pool was divided into two blocks and within each block we planted communities containing 1, 2 or 4 species realizing all possible compositions. In each block, community composition was replicated twice for monocultures, four times for 2-species and 12 times for 4-species-mixtures. Because the distance between quadrants varied and neighbours in opposite positions were growing further away than neighbours beside each other, we arranged species positions differently in replicates of 2-species and 4-species mixtures. We established six control pots for each species in which only one quadrant was planted with two individuals, i.e. there were no competitors in the neighbour quadrants.

CMN treatment and nutrient tracing

The polyoxymethelene pots were 23x23x12 cm in size and separated into four quadrants (11x11x12 cm) by double walls (Fig. 2). The walls were 1 cm apart and had windows covered by 37µm nylon mesh. Two stainless steel wires were installed within the gap of each double wall running across the pots from each side. Hyphal connections between quadrants could be cut by pulling the wire up and down. This treatment was repeated once per week for the duration of the experiment. The pots for the corresponding control treatment had no installed wires allowing unconstrained hyphal growth between quadrants.

To trace phosphorus uptake from neighbouring quadrants we applied two different P radio isotopes to each pot. We applied 2 ml (0.5MBq) of ^{32}P- or ^{33}P-$H_3PO_4^-$ solution to one pot quadrant and the other tracer to another quadrant of the same pot. We accounted for distance differences between neighbouring quadrants and varied label positions between composition replicates. Due to the short half-life of the radio isotopes we repeated the P tracer application 5 times during the course of the experiment.

Plant growth and nutrient uptake

We measured the number of leaves and shoots and the width and length of the three largest leaves of each individual in intervals of up to three weeks. Shoot biomass was then estimated using species-specific allometric equations that were obtained by linear regressions of log-transformed harvested shoot biomass vs. log-transformed plant traits (Table 1). Biomass from the first and the last harvest was dried at 80 °C and weighed. To determine total phosphorus, ^{32}P and ^{33}P pools in plants, fresh leaf biomass subsamples of the individuals from one quadrant were combined and ashed for 3 hours at 600 °C. The ash was dissolved in 2 mL hot 1 M sulphuric acid and 5 mL ddH_2O before the solution was filtered (MN 615, Macherey-Nagel GmbH & Co. KG, Düren, Germany). ^{32}P and ^{33}P concentrations were determined by liquid scintillation counting (TriCarb, QuantaSmart, Ultima Gold LLT scintillation cocktail, Perkin Elmer, Waltham, MA). P concentrations were measured by automated colorimetry (San^{++}, Skalar Analytical B.V., Breda, Netherlands).

Setup and growing conditions

Prior to experimental set up, seeds (Appels Wilde Samen GmbH, Darmstadt, Germany) of all species were germinated and kept for 8 weeks. In the pots the seedlings were grown on a mixture of 50 % Quartz sand (BR Bauhandel AG, Rümlang, Switzerland) and 50 % nutrient-poor soil from an extensively grazed calcareous grassland in north-western Switzerland. The soil is a Rendzina-type silty clay loam (30 % clay, 56 % silt, 14 % sand) characterized by a 10-15 cm neutral to slightly basic top soil (pH ≈ 7-8) and a calcareous base. The soil was air dried and manually sieved through 4 mm and filled on top of a gravel (8 mm) layer into the quadrants of each pot. The gap between quadrants was filled with quartz sand in order to facilitate wire movement. Eight weeks after transplantation we started applying 5 ml of ¼ Hoagland solution to the soil of each quadrant on a weekly base. In order to ensure phosphorus limitation the Hoagland solution did not contain phosphorus.

Temperatures were kept between 13 °C and 23 °C during the day and between 11 °C and 20 °C at night. Humidity was constantly kept between 40 and 50 % and lights were automatically switched on between 6:00 and 22:00 when light intensity was below 20 klx. Each pot received 150 ml of water, three times per week.

Data analysis

We tested for effects of CMN connectedness and species richness on plant shoot biomass, root biomass, relative growth rate (RGR) and shoot P pools at individual (quadrant) and community level using analysis of variance (aov function of R 3.2; http://www.r-project.org). Data was log-transformed to achieve a normal residual distribution. Quadrant-level models contained the terms summarized in Table 2 (Appendix). Within-pot terms were dropped for analysis at community level.

Effects of species richness on community biomass, nutrient contents, and nutrient tracer uptake in plant communities were partitioned into complementarity and selection effects according to Loreau & Hector (2001). Effects of CMN connectedness on selection and complementarity effects were analyzed using the following model terms, which are a subset of model terms summarized in Table 2 (Appendix): (1) Pool, (2) log (species richness), (3) pool x log (species richness), (4) CMN, (5) pool x CMN, (6) log (species richness) x CMN, (7) community composition, (8) CMN x community composition.

Differences in shoot biomass, root biomass and RGR (calculated from biomass estimates) between competition-free controls and monocultures were determined using analysis of variance with the following terms: (1) Pool, (2) block, (3) number of individuals, (4) species, (5) number of plants x species, (6) harvest, (7) harvest x terms (3)-(5).

To determine the movement of P nutrient tracers we analyzed the amount of nutrient tracer in labelled plants from quadrants receiving isotope application in relation to tracer in the whole community.

Results

Competition-free (1-quadrant) controls

Plants in competition-free controls produced significantly more shoot (P=0.001, $F_{1,61}$=487.3) and root biomass ($F_{1,60}$=59.2, P=0.001) (Fig. 4 a, b) and had significantly greater root-shoot ratios ($F_{1,60}$=14.4, P=0.001) than plants growing in monoculture quadrants, regardless of species identity. Relative growth rates (RGR), calculated from allometric biomass estimates, tended to be greater in competition-free controls during the first growing period ($F_{1,59}$=2.9, P=0.1) resulting in relatively steeper growth curves (Fig. 3). Consequently, at last harvest, plants grown without competition produced 2.3 g shoot biomass on average and plants in monocultures 0.8 g. Average root biomass was 3.3 g for plants from competition-free controls and 1.5 g for plants in monocultures.

Shoot and root biomass as well as RGRs of plants grown without competition were unaffected by the CMN treatment (Fig. 3, Fig. 4 a, b).

Community-level effects

Community species richness affected neither community shoot and root biomass nor community P pools. The CMN treatment significantly decreased community shoot biomass ($F_{1,128}$=8.9, P=0.004) in particular at last harvest ($F_{1,128}$=6.9, P=0.01) (Fig. 5). CMN effects were dependent on community species richness ($F_{1,128}$=4, P=0.05, CMN x log (species richness)) (Fig. 5) and community composition ($F_{20,128}$=2.3, P=0.003, CMN x composition). Plants in monocultures were unaffected by the CMN treatment whereas plants in 2-species ($F_{1,70}$=6.9,

P=0.01) and 4-species communities ($F_{1,41}$=9.1, P=0.005) had reduced shoot biomass (Fig. 5). Community root biomass tended to be higher ($F_{1,122}$=3.3, P=0.07) when CMNs were disconnected (Fig. 5) and consequently community root-shoot ratios were higher ($F_{1,122}$=7.19, P=0.009). Total shoot P pools in the community as well as ^{33}P or ^{32}P uptake were unaffected by the CMN treatment (Fig. 5).

Individual-level effects

Species richness had no effects on individual biomass production or shoot P pools (Fig. 4 a, b). The disconnection from CMNs significantly reduced individual shoot biomass ($F_{1,128}$=5.43, P=0.022) in particular at last harvest ($F_{1,128}$=6.87, P=0.01) (Fig. 4 a, b, show mean of all harvests). However, RGRs, derived from biomass estimates, were unaffected by the CMN treatment during the first and second growing period (Fig. 3). Individual root biomass tended to increase with the disconnection from CMN ($F_{1,122}$=3.65, P=0.059) (Fig. 4 a, b). Hence, root-shoot ratios were significantly greater ($F_{1,122}$=7.52, P=0.008) with the CMN treatment. Shoot P pools tended to be reduced when plants were excluded from the CMN ($F_{1,130}$=4.16, P=0.07) (Fig. 4 a, b) with greater decreases in monocultures than in mixtures ($F_{1,12}$=10, P=0.009, CMN x monoculture vs. mixture) and species-specific differences in monocultures ($F_{7,128}$=3.36, P=0.07, CMN x species in monoculture) (Fig. 4 a, b). Individual ^{33}P and ^{32}P uptake were unaffected by the CMN treatment.

Species interactions

Generally there were no net effects of species richness on shoot biomass, root biomass or community P pools (Fig. 5). Complementarity effects on shoot biomass were overall positive but significantly decreased when CMN were cut ($F_{1,11}$=5.7, P=0.04), whereas selection effects were unaffected by the treatment (Fig. 6). Complementarity and selection effects for root biomass were not affected by the CMN treatment. For community P pools selection effects did

not change whereas complementarity increased significantly ($F_{1,9}$=7.6, P=0.02) (Fig. 6). 2-species mixtures tended to become more even with CMN disconnection ($F_{1,70}$=3.4, P=0.07) whereas evenness in 4-species mixtures was unaffected by the CMN treatment. We found no effects of CMN on root biomass or P pool distribution in either community (data not shown). Standard deviation from mean shoot biomass, mean root biomass and mean shoot P pools in monocultures and conspecifics of 2-species mixtures were unaffected by the disconnection from CMN (data not shown).

The CMN treatment did not affect the relative ^{33}P or ^{32}P amount in position of tracer application (Data not shown).

Discussion

Here we altered the connectedness of plants through AMF networks (CMN) in order to test the hypothesis that the reduction of nutrient mobility by reduced AMF connectedness will reduce competition between plants. The prevention of hyphal growth from host plants into neighbouring quadrants and potential formation of AMF networks had substantial effects on plant growth and nutrient pools in plant communities. Shoot biomass and phosphorus pools were significantly reduced whereas root biomass increased with disconnection from hyphal networks. These results suggest that the connectivity of plants via a common mycorrhizal network (CMN) is beneficial for plants. Plants grown without neighbours produced significantly more shoot and root biomass, confirming the presence of plant-plant competition when plants grow in communities. Shoot and root growth were not affected by the disconnection from the CMN in competition-free controls. These results illustrate that the mobility of nutrients among plants via CMN in plant communities affected plant-plant competition rather than the alteration of nutrient availability per se. Besides the physiological benefits provided to plants by the CMN connection, we found that the complementarity of species measured in shoot biomass was reduced with the treatment. Species mixtures decreased

community biomass when plants were disconnected from the CMN. Conversely, monocultures remained unaffected, thus implying that the benefits from mixtures are caused by species complementarity. Plants in monocultures showed greatest decreases in P pools but with species specific differences. These results reflect species specific CMN responses and may explain the decrease in complementarity for P pools when CMN were absent.

We observed that the disconnection of plants from CMNs reduced species complementarity in mixtures and nutrient uptake in monocultures, rather than reducing inter-specific or intraspecific competition. The benefits of CMN connection for plant growth in mixtures and monocultures potentially outweighed effects of reduced nutrient mobility on plant-plant competition.

CMN mediated plant-plant competition

It has been shown that AMF can alter individual plant growth and greatly affect the outcome of plant-plant competitive interactions, particularly if hosts differ in their mycorrhizal response (Zobel and Moora 1995, van der Heijden et al. 1998, Scheublin et al. 2007, Wagg et al. 2011). However, this mutualism may influence plant-plant competitive outcomes differently. For instance, it has been suggested that AMF can relax competitive interactions and thereby promote coexistence by favoring competitively inferior individuals (Wagg et al. 2011). The benefit of less competitive and AMF dependent plants when connected to a CMN may be a consequence of nutrient transfer from a more competitively dominant plant towards subordinate plants through CMNs (Read 1997, Simard and Perry 1997, Urcelay and Díaz 2003, Kiers et al. 2011, Walder et al. 2012). On the other hand AMF may favor dominant mycorrhizal-dependent plant species and thereby increase the performance of strong competitors, and may ultimately lead to competitive exclusion (West 1996; Allen et al. 2003; Urcelay & Diaz). For both scenarios it could be shown that plant and fungal diversity play an important role (Wagg et al. 2011, Walder et al. 2012). Here we tested effects of reduced nutrient mobility through

AMF hyphal growth and found that the connectedness of plants in a CMN did indeed balance plant-plant competition.

The role of nutrient transfer via CMN

Interestingly, we could not find indications of decreased movement of P nutrient tracer between plants when hyphal growth was restricted. Plants that were disconnected from the CMN had significantly increased root growth. This may be a result of compensatory growth in order to obtain nutrients otherwise available through external hyphae growth. Generally, nutrient uptake by external hyphae is considered to be less important to the plant when root densities are high because enough resources can be acquired from the plant's inherent depletion zone (Koide 1991, Ayres et al. 2006). However, here it was not colonization per se that led to increased root-shoot ratios but rather the limitation of hyphal growth into neighbouring quadrants, confirming the importance of rhizosphere extension through external hyphae growth for nutrient acquisition. The compensation for reduced hyphal growth by increased root growth may explain why P nutrient tracer movements were unaffected by the disconnection from the CMN. However this would require that the P tracer travelled far enough by diffusion in order to be available in other quadrants of the pot.

In this study, we intended to reduce nutrient mobility between plants by cutting their hyphal connections. However tracer isotopes showed that for at least phosphorus the mobility was more or less unaffected by this intervention, potentially due to increased root growth. Due to the strong effects on above- and below-ground biomass we observed with the treatment we have to assume that hyphal connections were interrupted. Whether the effects observed are a result of nutrient mobility between plants, potentially also nitrogen, or whether the effects are caused by other CMN mediated effects between plants remains speculative.

Conclusions

Here we found that CMN disconnection had negative effects on individual plant growth and species complementarity in mixtures which was potentially caused by decreased nutrient mobility between plants. We did not find that CMN disconnection and an associated reduction of nutrient mobility between plants reduced their competitive interactions. As opposed to the study by Wilberts et al. (2013) we manipulated nutrient mobility via a biotic interaction that generally contributes to plant nutrition and nutrient mobility in soils. However, AMF hyphae are not an unbiased means of nutrient transportation (Kiers et al. 2011, Fellbaum et al. 2014). AMF mediated nutrient transport can be directed by favoring certain host plants over others and this can affect competitive outcomes (Scheublin et al. 2007, Kiers et al. 2011, Walder et al. 2012). To test the effects of reduced nutrient mobility on plant competition, the use of CMN as mean of nutrient transport regulation may be questionable and results obtained more difficult to interpret. However, besides abiotic factors that affect soil nutrient mobility, AMF networks are potentially the most important biotic factor that have an effect on soil nutrient mobility in natural systems (Walder et al. 2012, Wagg et al. 2015), which highlights the implications of our results. The maintenance of AMF fungal networks plays an important role in sustainable agriculture because of its positive effects on plant productivity (Rooney et al. 2009) and known implications for plant community structure (Grime et al. 1987, van der Heijden et al. 1998, van der Heijden and Horton 2009). Our results show that the destruction of such networks, which is a common consequence of conventional farming practices, cannot solely reduce individual and community productivity by eliminating any direct positive effects of the mutualism but also indirectly affects plant community structure by altering complementary resource use and facilitation.

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Table 1: Allometric equations for eight study species. Allometric coefficients derive from log linear regressions of biomass at harvest and plant trait measurements.

Species	Allometric equation (x= shoot biomass estimate)
Plantago lanceolata	-8.085+1.5173*log(leaf number)+1.075*log(leaf length)+0.783*log(leaf width)
Prunella grandiflora	-7.404+1.423*log(leaf number)+1.386*log(leaf length)
Hierarcium pilosella	-4.99+1.09*log(leaf number)+0.599* log(leaf width)
Plantago media	-6.045+0.125*log(leaf number)+0.876*log(leaf length)+2.591*log(leaf width)
Holcus lanatus	-7.394+1.11*log(leaf number)+0.97*log(leaf length)-0.254*log(leaf width)
Festuca pratensis	-7.38+1.276*log(leaf number)+0.639*log(leaf length)-0.802*log(leaf width)
Anthoxanthum odoratum	-6.610+1.246*log(leaf number)+0.572*log(leaf length)+0.673*log(leaf width)
Bromus erectus	-7.506+1.237*log(leaf number)+0.953*log(leaf length)+0.215*log(leaf width)

Figure 1: An illustration of hypothetical effects caused by the disconnection of plants from AM fungal networks (CMN) in the presented experimental setup. At individual level, reduced competition may increase growth of weak competitors. At community level selection effects may be reduced and evenness increased in communities of heterospecifics. Hypothetically reduced intraspecific competition may reduce size inequality in monocultures. The uptake of tracer isotope of plants in position of tracer application may be greater when CMN are absent.

Figure 2: Design of custom-build pots with double walls and installed stainless steel wires for CMN treatment. Each wall has a window covered by 37µm nylon mesh. The wires can be moved up and down in order to cut hyphal connections from plants between quadrants. For control pots no wires were installed.

Figure 3: Mean estimated shoot biomass of plants (2 individuals) in species pool 1 and species pool 2 when grown without competition, with intraspecific competition in monocultures or interspecific competition in 2-species and 4-species mixtures over time (DOY: Day of Year since 1st January 2012). Data derives from the first (240 - 340 DOY) and the last growing period (430 - 500 DOY). Biomass estimates derive from plant trait measurements at six observation times in the first growing period and four observation times in the last growing period. Solid lines represent control pots; dashed lines represent pots with CMN treatment. P = time of 32P/33P isotope application; HL = start of Hoagland fertilizer application.

Figure 4 a,b: Mean (of two harvests) shoot biomass, root biomass and P pool of species in pool 1 (a) and species in pool 2 (b) when grown without competition (1-quadrant), in monocultures, 2-species or 4-species mixtures. *Festuca pratensis* (F.p), *Holcus lanatus* (H.l),

Plantago lanceolata (P.l) *Prunella grandiflora* (P.g), *Anthoxantum odoratum* (A.o), *Bromus erectus* (B.e), *Plantago media* (P.m), *Hierarcium pilosella* (H.P). White bars or symbols represent control pots; grey bars or symbols biomass or P pools in pots with CMN treatment. Error bars show standard errors of the mean.

Figure 5: Mean (of two harvests) community shoot biomass, root biomass and P pools of competition-free controls (1-quadrant), monocultures, 2-species and 4-species mixtures of species in pool 1 and pool 2. White bars represent control pots; grey bars represent pots with CMN treatment. Error bars show standard errors of the mean.

Figure 6: Mean selection and complementarity effects (of two harvests) for shoot biomass, root biomass and shoot P pools in mixed communities of species pool 1 and species pool 2 when grown with (grey bars) or without (white bars) CMN treatment. Error bars show standard errors of the mean.

Figure 1

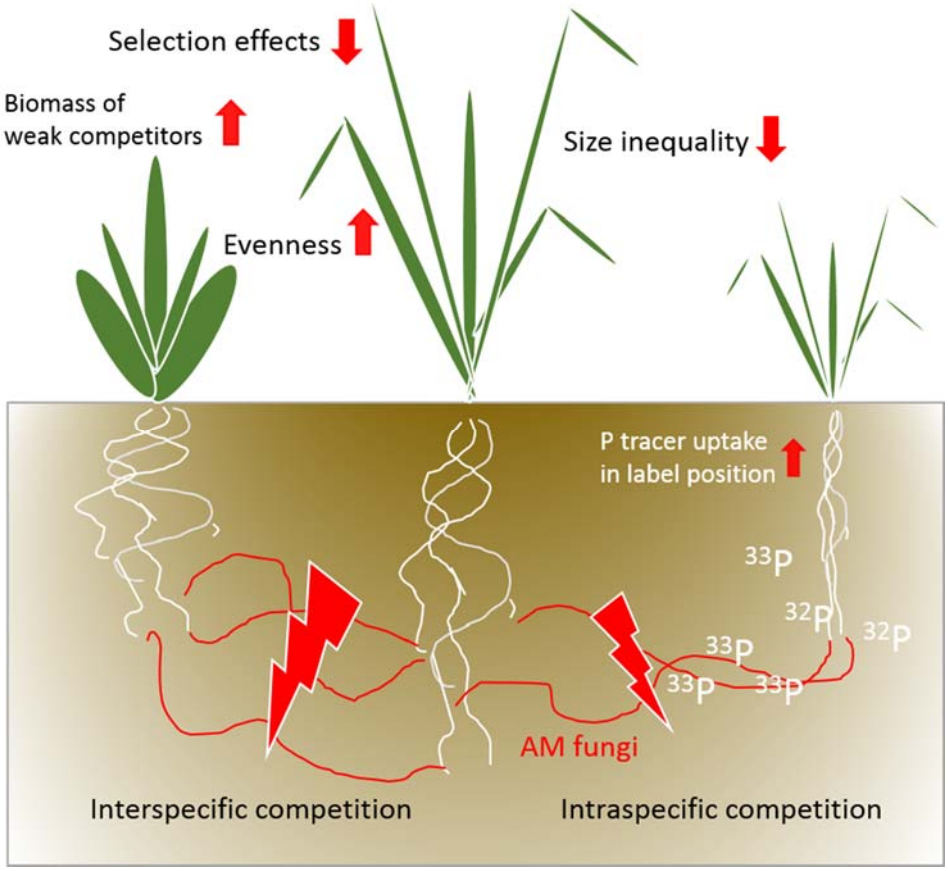

Selection effects
Biomass of
weak competitors
Evenness
Size inequality
P tracer uptake
in label position
33P
32P
32P
33P
33P
33P
AM fungi
Interspecific competition
Intraspecific competition

Figure 2

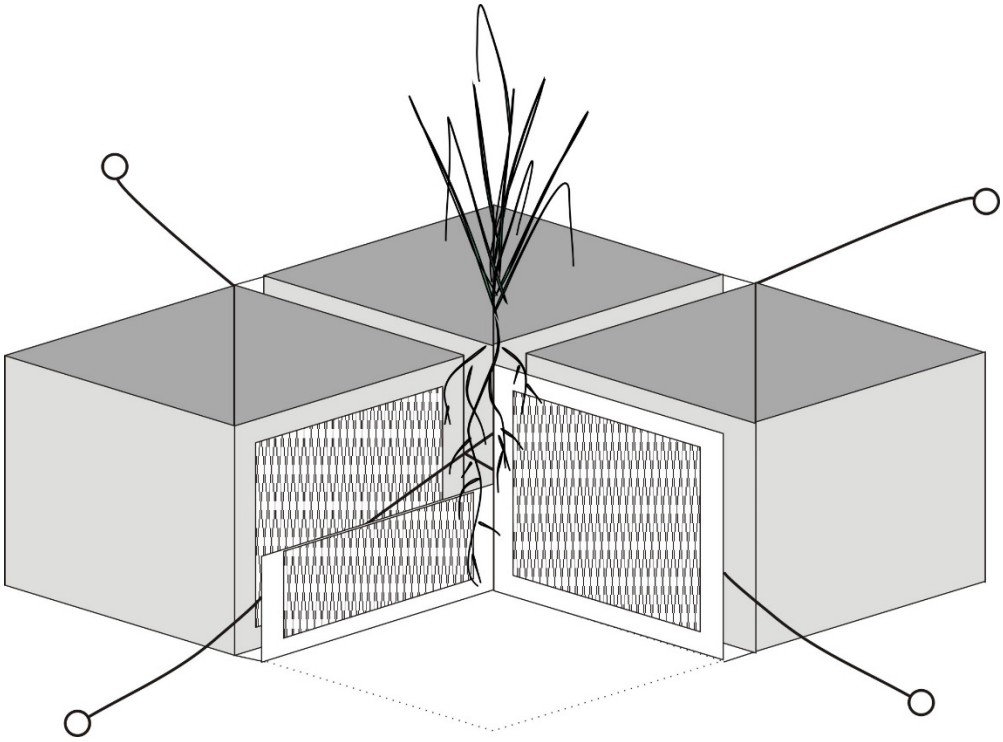


Figure 3

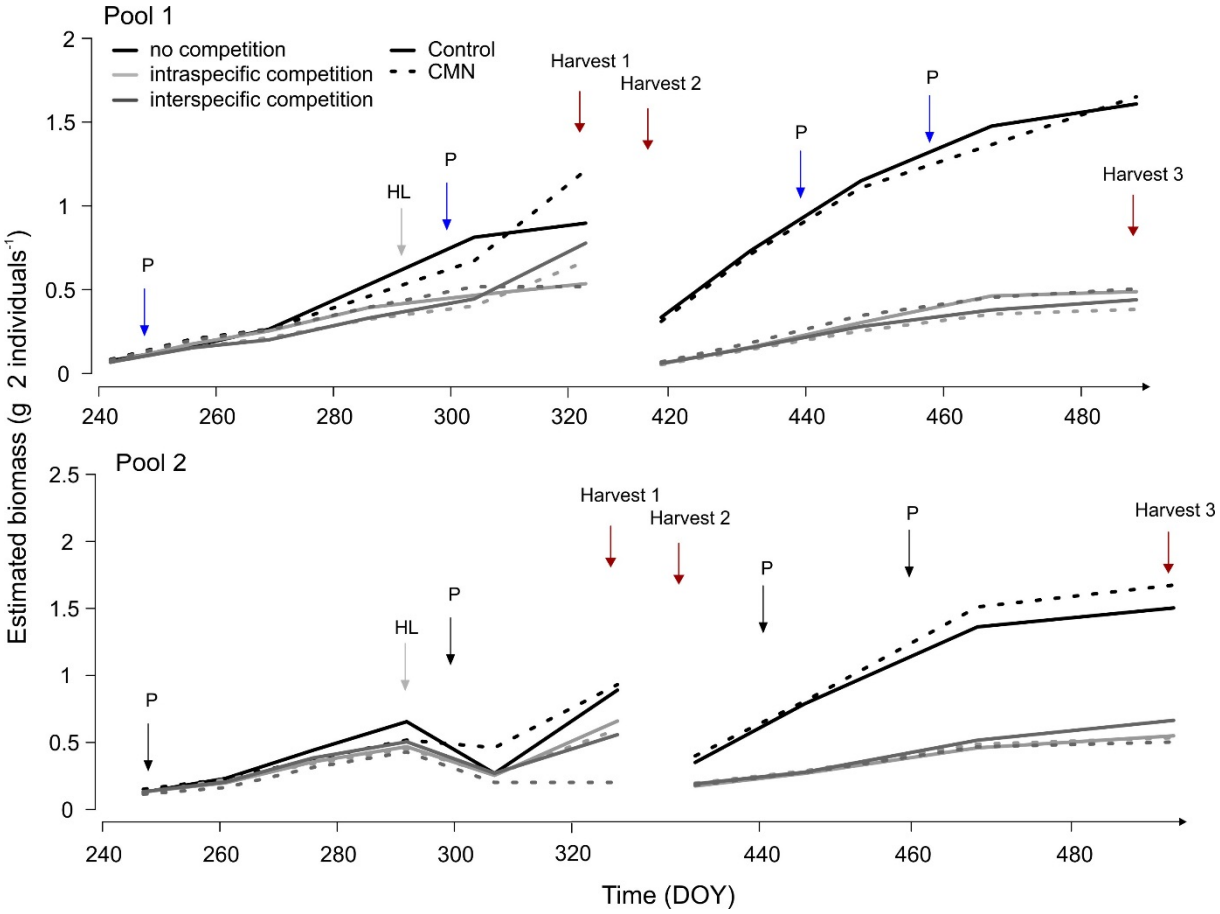

Pool 1
no competition
intraspecific competition
interspecific competition
Control
CMN
Harvest 1
Harvest 2
Harvest 3
P
HL
Pool 2
Estimated biomass (g 2 individuals-1)
Time (DOY)

Figure 4 a

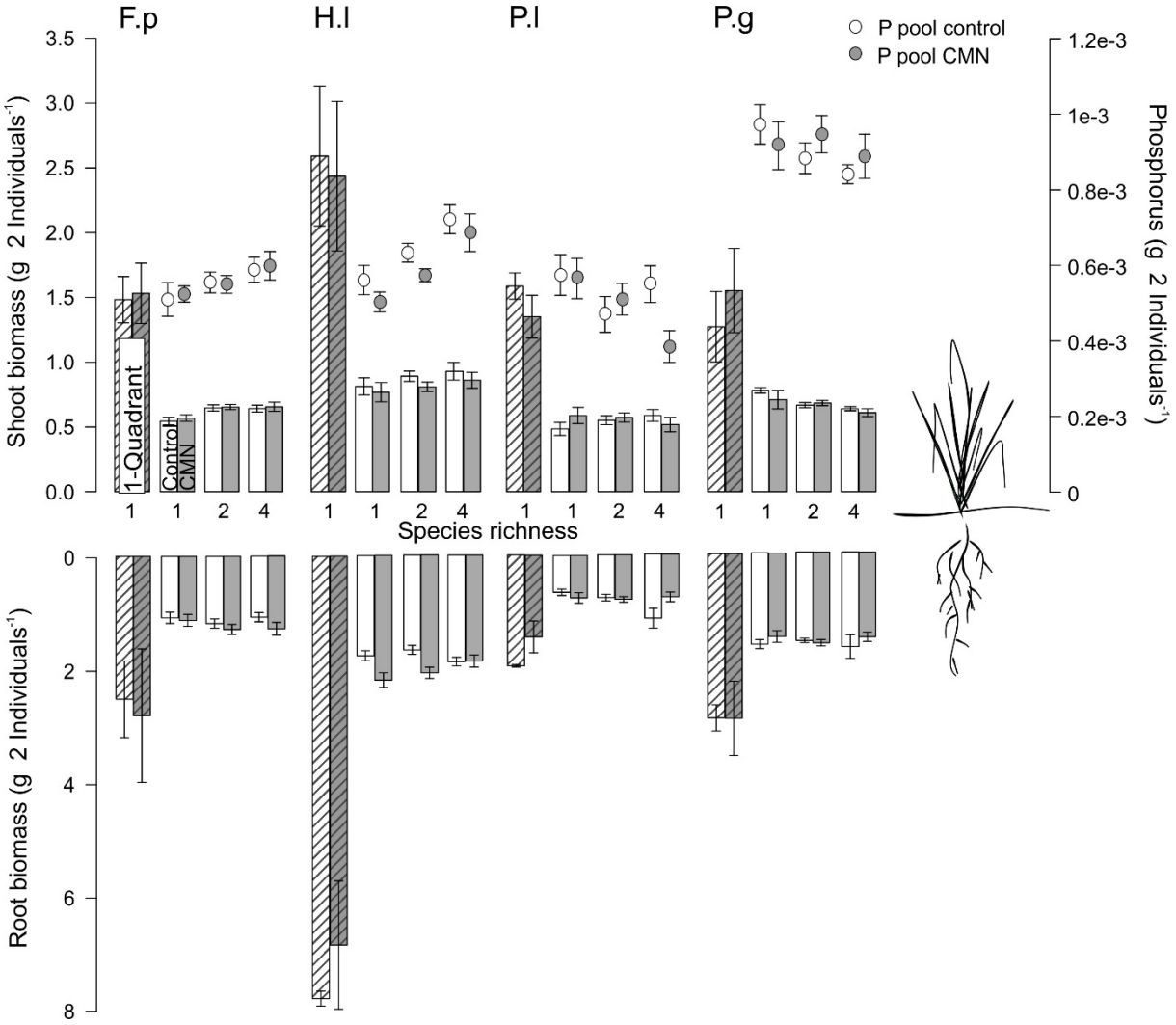

F.p
H.l
P.l
P.g
P pool control
P pool CMN
Shoot biomass (g 2 Individuals-1)
Root biomass (g 2 Individuals-1)
Phosphorus (g 2 Individuals-1)
1-Quadrant
Control
CMN
Species richness
1 1 2 4 1 1 2 4 1 1 2 4 1 1 2 4
0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5
0 2 4 6 8
0 0.2e-3 0.4e-3 0.6e-3 0.8e-3 1e-3 1.2e-3

Figure 4 b

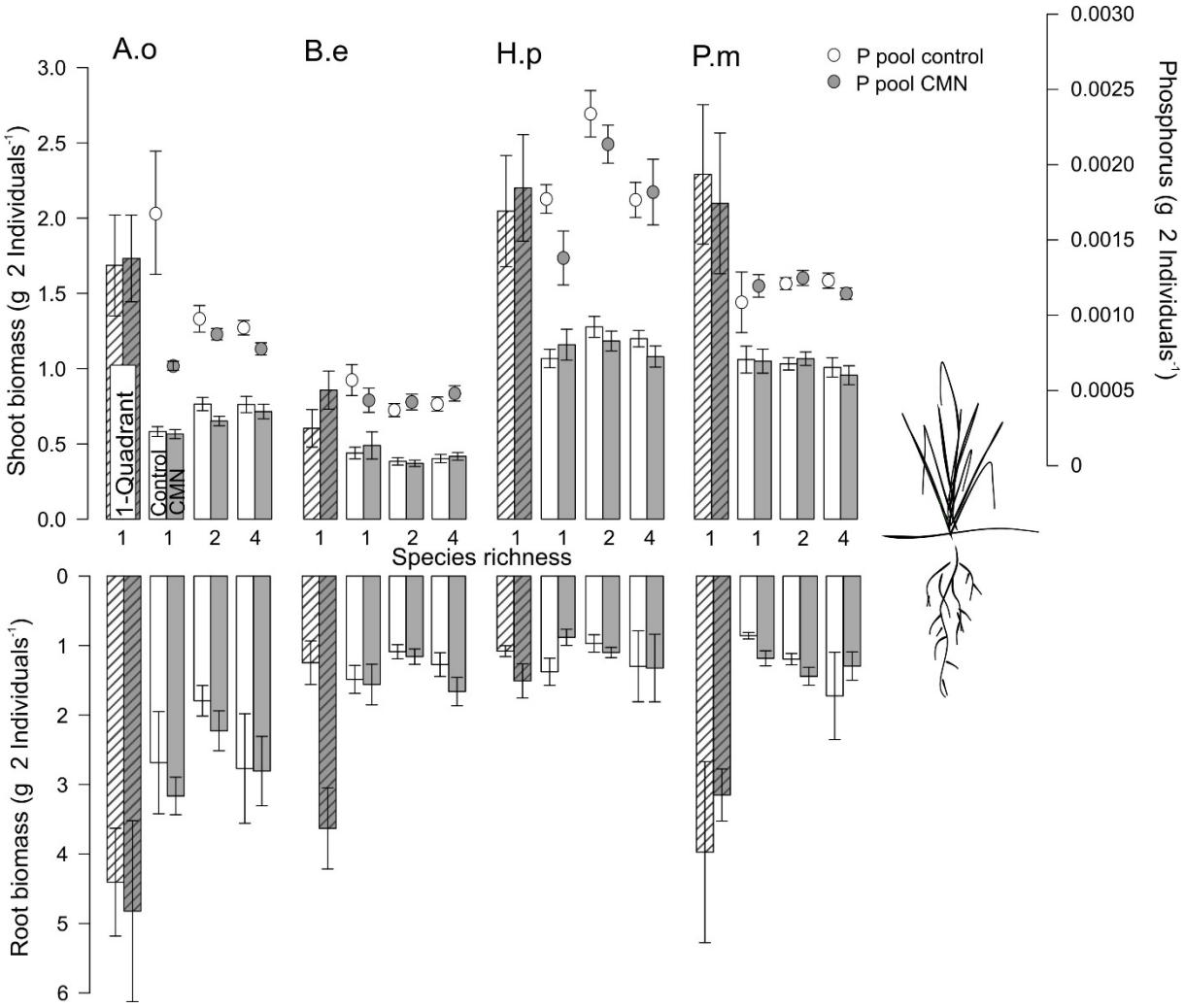

A.o
B.e
H.p
P.m
P pool control
P pool CMN
Shoot biomass (g 2 Individuals^{-1})
Root biomass (g 2 Individuals^{-1})
Phosphorus (g 2 Individuals^{-1})
1-Quadrant
Control
CMN
Species richness
1 1 2 4 1 1 2 4 1 1 2 4 1 1 2 4
0.0 0.5 1.0 1.5 2.0 2.5 3.0
0 1 2 3 4 5 6
0 0.0005 0.0010 0.0015 0.0020 0.0025 0.0030

Figure 5

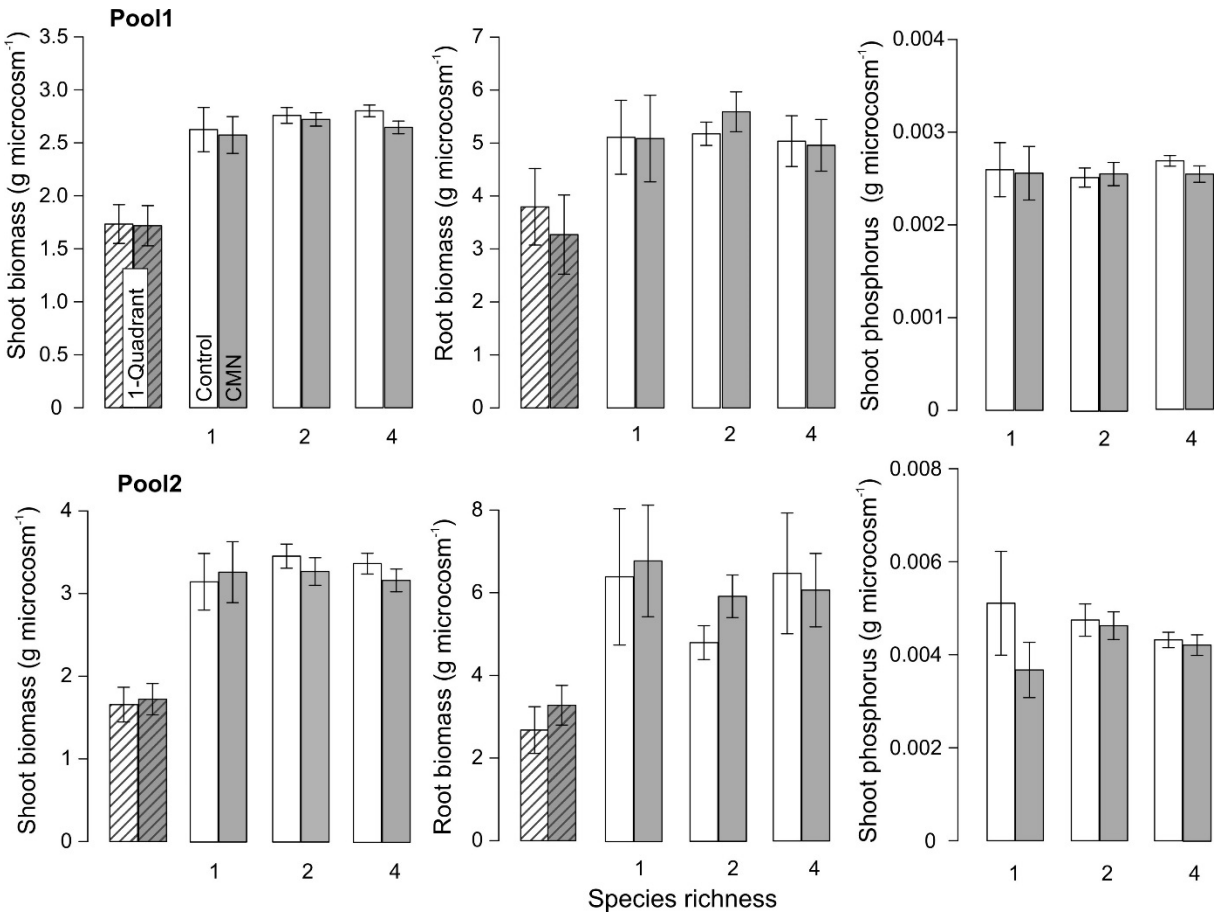
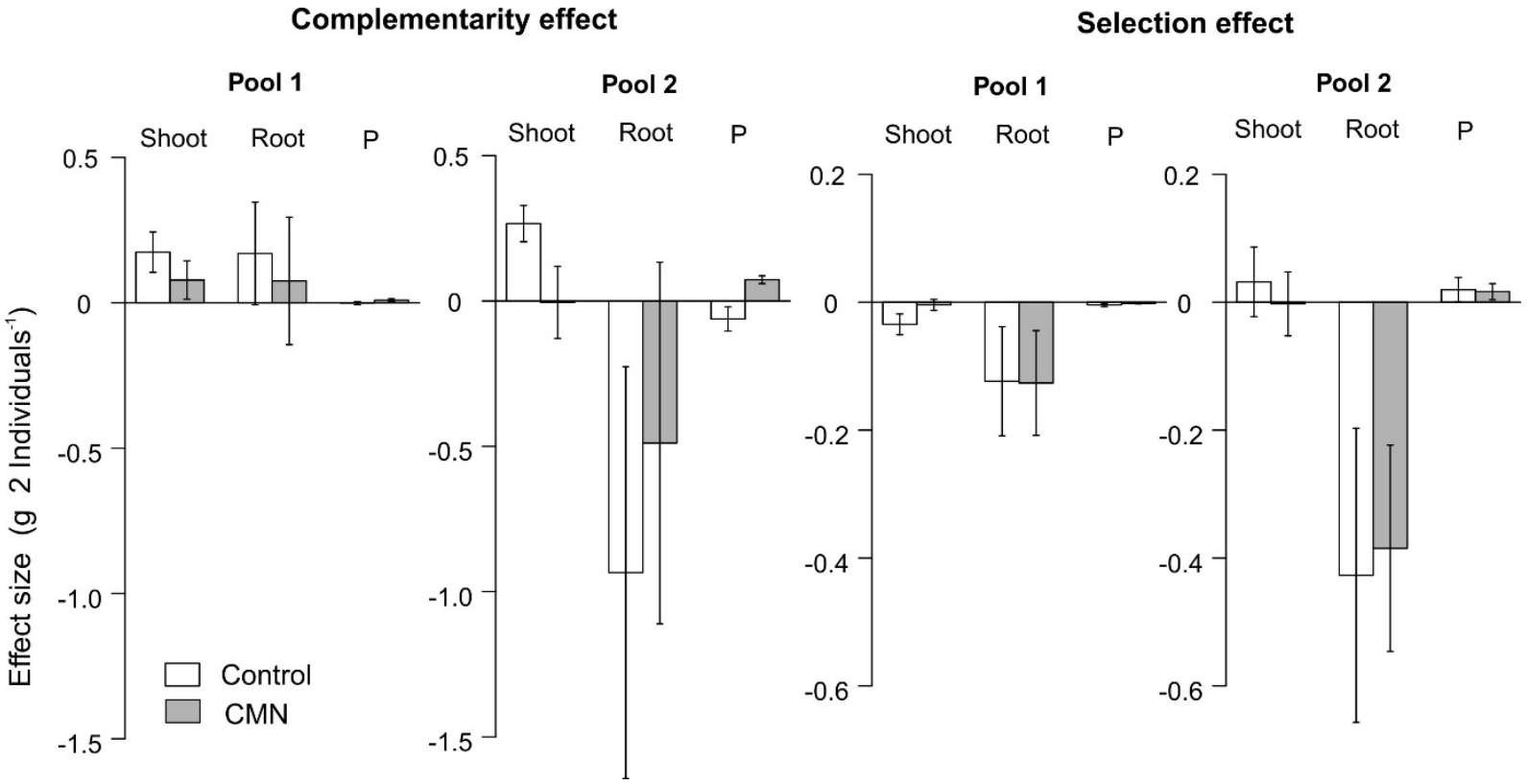

Pool1
Pool2
Shoot biomass (g microcosm-1)
Root biomass (g microcosm-1)
Shoot phosphorus (g microcosm-1)
1-Quadrant
Control
CMN
Species richness

Figure 6


Complementarity effect
Selection effect
Pool 1
Pool 2
Pool 1
Pool 2
Shoot
Root
P
Shoot
Root
P
Shoot
Root
P
Shoot
Root
P
Effect size (g 2 Individuals-1)
0.5
0
-0.5
-1.0
-1.5
0.5
0
-0.5
-1.0
-1.5
0.2
0
-0.2
-0.4
-0.6
0.2
0
-0.2
-0.4
-0.6
Control
CMN

Appendix

Table 2*:* Structure and results of analysis of variance

					Individual level				Community level									
Level	Term	df	Error	ddf	Shoot biomass (log)	Root biomass (log)	Root: Shoot ratio (log)	P (log)	Shoot biomass (log)	Root biomass (log)	Root: Shoot ratio (log)	P (log)	E 1/D (asin)	SD Shoot biomass (log)	SD Root biomass (log)	SD P (log)	32P label pos. (asin)	33P label pos. (asin)
Block																		
	Pool	1	Block	2	n.s.	n.s.	n.s.	*	(*)	n.s.	n.s.	*	*	n.s.	*	n.s.	n.s.	n.s.
	Residual (Block)	2																
Composition																		
	Species richness (SR)																	
	log SR	1	mixture composition	11	n.s	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	(*)	n.s.	n.s.	n.s.	n.s.
	monoculture vs. mixture	1	mixture composition	11	n.s.	n.s.	n.s.	n.s.	—	—	—	—	—	—	—	—	—	—
	Residual																	
	monoculture species	7	mixture composition	11	n.s.	(*)	***	n.s.	—	—	—	—	—	(*)	n.s.	*	**[1]	***[1]
	mixture composition	11																
Pot																		
	CMN	1	Residual (Pot)	128	*	(*)	**	(*)	**	(*)	**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Composition x CMN																	
	CMN x Species richness																	
	CMN x log SR	1	CMN x mixture composition	12	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	—	—	—	n.s.	n.s.
	CMN x monoculture vs. mixture	1	CMN x mixture composition	12	n.s.	n.s.	n.s.	**	—	—	—	—	—	**	n.s.	n.s.	—	—
	CMN x residual composition																	
	CMN x monoculture species	7	CMN x mixture composition	12	n.s.	n.s.	n.s.	**	—	—	—	—	—	*	n.s.	n.s.	n.s.[1]	n.s.[1]
	CMN x mixture composition	12											n.s	n.s.	n.s.	n.s.	n.s.	n.s.
	Residual (Pot)	128																

Population (Species x Pot, mixtures only)																	
Species	6	Residual (Population)	201	***	***	***	***	—	—	—	—	—	(*)	*	***	—	—
Species x Composition																	
Species x log SR	6	Residual (Species x Composition)	6	n.s.	n.s.	n.s.	(*)	—	—	—	—	—	—	—	—	—	—
Residual (Species x mixture composition)	6																
Species x Composition x CMN																	
Species x CMN	6	Residual (Population)	201	n.s.	n.s.	n.s.	n.s.	—	—	—	—	—	n.s.	n.s.	n.s.	—	—
Species x CMN x log SR	6	Residual (Species x Composition x CMN)	6	n.s.	n.s.	n.s.	n.s.	—	—	—	—	—	—	—	—	—	—
Residual (Species x mixture composition x CMN)	6																
Residual (Population)	201																
Harvest x Population																	
Harvest																	
Harvest x Pool		Harvest x Block															
Harvest x Block																	
Harvest x log SR	1	Harvest x mixture composition	12	n.s.	—	—	—	n.s.	—	—	—	n.s.	—	—	—	n.s.	(*)
Harvest x monoculture vs. mixture	1	Harvest x mixture composition	12	n.s.	—	—	—	—	—	—	—	—	—	—	—	—	—
Harvest x monoculture species	7	Harvest x mixture composition	12	n.s.	—	—	—	—	—	—	—	—	*	—	—	*[1]	***[1]
Harvest x mixture composition	12																
Harvest x CMN	1	Harvest x Pot	130	n.s.	—	—	—	n.s.	—	—	—	n.s	n.s.	—	—	n.s.	n.s.
Harvest x CMN x log SR	1	Harvest x CMN x mixture composition	12	n.s.	—	—	—	*	—	—	—	*	—	—	—	n.s.	n.s.
Harvest x CMN x monoculture vs. mixture	1	Harvest x CMN x mixture composition	12	*	—	—	—	—	—	—	—	—	**	—	—	—	—
Harvest x CMN x monoculture species	7	Harvest x CMN x mixture composition	12	n.s.	—	—	—	—	—	—	—	—	n.s.	—	—	n.s.	n.s.
Harvest x CMN x mixture composition	12	Harvest:Pot	130	n.s.	—	—	—	*	—	—	—	—	n.s.	—	—	—	—

Harvest x Pot	130															
Harvest x Species	6	Harvest x Species x Pot	201	***	—	—	—	—	—	—	—	—	(*)	—	—	—	—
Harvest x Species x log SR	6	Harvest x Species x composition	6	n.s.	—	—	—	—	—	—	—	—	—	—	—	—	—
Harvest x Species x composition	12																
Harvest x Species x CMN	6	Harvest x Species x Pot	201	n.s.	—	—	—	—	—	—	—	—	n.s.	—	—	—	—
Harvest x Species x CMN x log SR	6	Harvest x Species x CMN x composition	6	n.s.	—	—	—	—	—	—	—	—	—	—	—	—	—
Harvest x Species x composition x CMN	6																
Harvest x Species x Pot	201																

Degrees of freedom are from the model including all observations from four blocks and two harvests when analyzed for effects on shoot biomass at individual or species level (up to 4 observations per pot). When we analyzed for effects on other variables or effects at community level model terms were reduced and degrees of freedom varied. E 1/D= Evenness index for 2-species and 4-species mixtures, SD= standard deviation from mean size in conspecifics of monocultures and 2 species mixtures, ^{32}P, ^{33}P label pos.= isotope amount in labelled plants (2 individuals) as proportion of total isotope amount in all plants of the pot.[1] here monoculture species refers to species in monoculture and mixture.

Chapter 3

Heteromyopia, a consequence of nutrient competition? Testing distance dependency of intra vs. interspecific resource competition

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Manuscript for publication with: E. Frossard[2], P. A. Niklaus[1,] P. Stoll[3]

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Abstract

Aims

It is controversial in plant ecology to which extent the spatial structure of plant communities can contribute to stable species coexistence. A competition distance relationship whereby conspecifics compete over longer distances than heterospecifics, but compete relatively less at shorter distances (heteromyopia) was proposed as co-existence stabilizing mechanism. Currently, there is no emprical evidence for the existence of heteromyopia and the driving mechanisms behind the process are unidentified. We are testing whether heteromyopia can be related to nutrient competition in plant neighbourhoods.

Methods

We investigated distance effects on nutrient uptake in plant neighbourhoods under controlled conditions in a glasshouse experiment and under natural conditions in a field study. In both studies we applied ^{33}P and ^{15}N nutrient tracers to a focal plant and measured tracer uptake in surrounding conspecific and heterospecific neighbours at increasing distances.

Important findings

We found that distance dependency of nutrient uptake differed between hetero- and conspecific neighbours under controlled glasshouse and natural field experimental conditions. This study suggests that heteromyopia is mediated through nutrient competition, although results are not always consistent and differ between nutrients and experimental conditions. In order to assess effects of differentiation in nutrient competition distances on species co-existence, we suggest further studies considering manipulation of spatial community structure.

Keywords

Plant competition, heteromyopia, spatial ecology, co-existence, distance

Introduction

Plant community diversity relies on mechanisms that prevent the exclusion of weak competitors. Using the ecological niche concept, we can explain that species may coexist because they differ in their habitat requirements or occupy a heterogeneous environment (for review see Barot & Gignoux 2004). Both, environmental heterogeneity and endogenous heterogeneity cause differentiation in species requirements, and are considered stabilizing mechanisms as they foster stable species co-existence. If differences in resource requirements are small and species share a homogenous environment, co-existence appears paradox but may arise from equalizing mechanisms such as life history trade-offs that reduce the fitness differences of otherwise similar competitors (Barot and Gignoux 2004). With a competition-colonization tradeoff co-existence is maintained because a weaker competitor may, due to higher reproduction rates or greater seed dispersal, colonise patches that are yet unoccupied by its rival (Levins and Culver 1971, Tilman 1994). Spatial community structure is crucial in this theory as it requires that patches are unoccupied. Plants interactions are restricted to their close neighbourhood (Stoll and Weiner 2000, Vogt et al. 2010). As a consequence of their sessile life style, the suitability of a location for survival, growth, and reproduction of plants is generally determined once their seed has dropped without the possibility to move to more favorable locations. Besides clonal growth (Eriksson 1986), seed dispersal is the most likely means for plants to interact over longer distances and escape an unfavorable environment. The consideration of space in plant competitive interactions appears intuitive and has received much attention in previous empirical (Tilman 1994, Rees et al. 1996, Milbau et al. 2007) and theoretical studies (Tilman and Kareiva 1997, Chesson 2000, Amarasekare 2003, Turnbull et al. 2007). The locally restricted nature of plant interactions causes distinct spatial structures in communities that distinguishes them from other organisms because individuals and species are often not as suggested for animal populations randomly distributed (Herben et al. 1999, Stoll

and Weiner 2000). It has been suggested that the spatial structure of plant communities promotes species co-existence (Murrell et al. 2002, Barot and Gignoux 2004). Aggregation of conspecifics is a commonly observed spatial pattern in plant communities that is generated by environmental patchiness or results from endogenous factors such as limited seed dispersal or clonal growth (Rees et al. 1996, Herben et al. 1999). In conspecific aggregates intraspecific interactions are more frequent and have been demonstrated to benefit weak competitors because they are protected from interactions with superior species (Weiner and Conte 1981, Rees et al. 1996, Murrell et al. 2001, Stoll and Prati 2001, Monzeglio and Stoll 2005, Mokany et al. 2008, Wassmuth et al. 2008, Porensky et al. 2012). Co-existence, however, is unstable as heterospecific interactions at the edge of clusters eventually give superior species the chance for invasion (Chesson and Neuhauser 2002). Spatial aggregation of conspecifics can delay competitive exclusion but it remains controversial as to which extent spatial patterns contribute to species co-existence in plant communities (Murrell et al. 2001, Rejmánek 2002).

Murrell & Law (2003) proposed a stabilizing mechanism in which spatial structure can promote stable co-existence of similar competitors in a homogenous environment. In an individual-based modelling approach, they considered competition as a neighbour type-specific function of space; a mechanism they introduced as "heteromyopia". Competitive interactions between plant individuals decrease with distance (Purves and Law 2002, Vogt et al. 2010). While previous modelling approaches that explored effects of spatial structure on competitive interactions widely assumed that interaction distances do not differ between species. Murrell & Law (2003), suggest that conspecific individuals interact over longer distances whereas heterospecifics interact relatively more at short distances. In this way, similar competitors could coexist because strong intraspecific competition over long distances would open gaps for others to occupy. Heteromyopia is a hypothetical mechanism yet empirical evidence for its importance in natural plant communities is scarce; further, the underlying mechanisms remain to be investigated.

Allelopathy or direct negative effects of heterospecific neighbours generally operate over short distances and may therefore reduce interaction distances with heterospecifics (Amarasekare 2003, Murrell and Law 2003), i.e. generate species-specific interaction distances as described for heteromyopia. Or, heteromyopia could be operating through indirect competition for resources between and within species. Light competition is in most cases restricted to the immediate neighbourhood thus heteromyopia is more likely related to competition for belowground resources. The extent to which plants can exploit soil resources is driven by their nutrient uptake efficiency, soil nutrient availability, and nutrient mobility (Marschner 1986). Conspecifics have similar exploitation strategies related to root morphology or chemical root compounds that may increase intraspecific- over interspecific interaction distances. The symbiosis of plants with arbuscular mycorrhizal fungi (AMF) can extend a plant host's exploitation area and affect plant competitive interactions (van der Heijden et al. 1998; Facelli et al. 2010; Wagg et al. 2011). Due to low host specificity, heterospecific and conspecific plants can be connected in common mycorrhizal networks (CMN) which potentially affect nutrient sharing and competitive interactions (Weremijewicz and Janos 2012) between plant hosts. Heteromyopia may be mediated through AMF networks by differential connectivity and nutrient competition between con- and heterospecific neighbours.

The aim of this study was to find out whether heteromyopia can be found in the nutrient uptake pattern of experimental and natural plant neighbourhoods. For this purpose we set up a microcosm glasshouse experiment and a field study. We applied nitrogen and phosphorus isotope tracers to the soil surroundings of a focal plant in a microcosm glasshouse experiment and in field plots and measured the uptake of nutrient tracer in conspecific and heterospecific neighbours.

We hypothesized, for both, the glasshouse experiment and the field study that nutrient uptake of neighbouring plants would be reduced with increasing distance to the focal plant. At

short distances we expected higher N and P tracer uptake in heterospecific than in conspecific neighbours, whereas conspecifics were expected to have higher uptake at greater distances. In other words, we expected that heterospecifics are better competitors at short distances than at long distances and vice versa. The combination of test results obtained under highly controlled glasshouse conditions with results collected under field conditions allowed us to infer conclusions about the existence of species specific interaction distances that would imply great importance for species co-existence.

Materials and Methods

Glasshouse experiment

We set up 9 microcosms with three different species compositions, assembled from five different study species. Each microcosm contained 31 plant individuals of three different species that were arranged on four circular distance zones around a center plant (Fig. 1). We applied ^{33}P and ^{15}N solutions in close proximity to the center plant to track nutrient movements to conspecific or heterospecific neighbours at different distances. Plants grew in microcosms for 45 weeks and were cut after 16 weeks and destructively harvested upon termination of the study after 45 weeks. We pooled individuals by species and distance and measured their aboveground biomass and ^{33}P concentrations at first harvest and ^{15}N concentrations at second harvest.

We used five temperate grassland species (*Plantago lanceolata*, *Plantago media*, *Hierarcium pilosella*, *Festuca pratensis*, *Holcus lanatus* (Appels Wilde Samen GmbH, Darmstadt, Germany)) that we arranged in three different compositions. Composition 1 contained *F.pratensis, H.pilosella, and P.lanceolata*. Composition 2 contained *H.lanatus, P. lanceolata, P.media* and composition 3 *F.pratensis, H.lanatus and P. lanceolata*. Each community composition was replicated three times, but the center plant differed between

replicates so that each species in the community occurred in the center once. The neighbourhood around the center plants was arranged in four circular distance zones 3.5 cm, 6 cm, 7 cm and 9 cm to the center (Fig. 1). At each distance zone we planted two individuals per species in alternating order resulting in 6 individuals per distance zone. In the outer distance zone we planted a total of 12 individuals with four individuals per species. This way each individual, except plants in the outer circle, which served as buffer to avoid boundary effects, was growing 3.5 cm away from its neighbours.

To track nutrient movements from the center plant to conspecific and heterospecific neighbours we applied ^{33}P and ^{15}N isotopes in immediate proximity to the center plant of each pot. We applied 2 ml (0.5MBq) of $H_3{}^{33}P\text{-}PO_4$ solution and 2 ml of 4 mM $(^{15}NH_4)_2SO_4$. Due to the short half life of ^{33}P isotopes we repeated the application during the course of the experiment. We cut plant shoots 4 cm above ground after 16 weeks and measured individual aboveground biomass and plant ^{33}P concentration as described in detail below. After 45 weeks we cut plants to ground level and measured ^{15}N concentrations (see below).

At the beginning of the study we transplanted eight week old seedlings into 3 L round pots that were lined with 20 mm drainage mats (Enkadrain, Schöllkopf AG, Rümlang, Switzerland) and filled with a mixture of 50 % quartz sand (BR Bauhandel AG, Rümlang, Switzerland) and 50 % nutrient-poor soil from an extensively grazed calcareous grassland in north-western Switzerland. The soil is a Rendzina-type silty clay loam (30 % clay, 56 % silt, 14 % sand) with 10-15 cm neutral to slightly basic top soil (pH ≈ 7-8) and calcareous base. The soil was air dried and manually sieved through 4 mm. Because plant growth stagnated after eight weeks, we started applying 30 ml of ¼ Hoagland solution to each pot and on a weekly base. The Hoagland solution did not contain phosphorus.

Field experiment

The field experiment comprised 48 circular plots with one out of eight study species located in the center. We applied ^{15}N and ^{33}P isotope tracers in close proximity to the center plant in order to track nutrient movements to conspecific and heterospecific neighbour individuals.

The presence and abundance of plant species on site were previously recorded (Zeiter et al. 2014) and we used the available data to identify and choose the four most abundant graminoids (*Bromus erectus, Festuca ovina, Helictotrichon pratense, Brachypodium pinnatum*) and forbs (*Thymus pulegioides, Prunella vulgaris, Helianthemum nummularium, Linum catharticum*). Other important criteria for the choice of the study species were that species are not forming associations with rhizobia and are not known to be particularly favored or disadvantaged by the symbiosis with mycorrhizal fungi.

For each of the eight study species we randomly chose 6 plots. Aboveground vegetation was removed to 1.5 cm distance around the center plant of each plot and bare ground was covered with weed control fabric to avoid regrowth of vegetation. Two weeks after plots were set up we applied 2 ml of tracer solution in immediate proximity to the center plant of each plot. The tracer solutions were prepared from $H_3{}^{33}P\text{-}PO_4$ solution (50 MBq) dissolved together with 4 g 99% enriched $(^{15}NH_4)_2SO_4$ in 150 ml water.

Weather conditions in the field were extremely dry during the experiment and resulted in significant mortality; as a result, the number of *Brachypodium pinnatum* and *Linum catharticum* samples was too small for further analysis and these species were thus excluded from the study. We further chose to harvest only three replicate plots per study species because that was the maximum number of plots for some species containing sufficient amount of samples to allow meaningful comparisons between the two competition types. For the analysis of competition type effects we categorized each species as conspecific or heterospecific neighbour of the center

plant. When more than three plots with the same center species contained sufficient sample size we chose the plots to be included in the study randomly. The number and identity of neighbour plants collected at different distances in the plots for each center species are summarized in Table 1.

After eight weeks we harvested the individuals of six study species from 18 plots. We harvested plants up to 30 cm to the center plant and recorded the distance before cutting. We measured individual shoot biomass and nutrient tracer concentration as described in detail below.

The study site is located within the plateau region in Switzerland (46°44′, 7°35) at 570 m a.s.l. and is designated military site. The vegetation is characterized by nutrient poor calcareous soils. The grassland is used extensively with one cutting per year. For the purpose of this study, an area of 100 m^2 has been excluded from any cutting regime from two months before and for the duration of the study. During the 2 months of the study average monthly temperatures were 21 °C and 19 °C and average precipitation 93 and 107 L m^{-2} for July and August respectively (www.thunerwetter.ch).

Nutrient tracer uptake

Samples from the glasshouse and the field study were oven dried for 48 hours at 80 °C and weighed. We obtained aqueous solutions from each sample by ashing them for 3 hours at 600 °C and dissolving the ash in 2 ml of hot 0.1 M sulphuric acid and 5 ml of ddH_2O. We filtered the solution (MN 615, Macherey-Nagel GmbH & Co. KG, Düren, Germany) and measured ^{33}P concentrations by liquid scintillation counting (TriCarb, QuantaSmart, Perkin Elmer, Inc., Waltham, MA, scintillation Cocktail: Ultima Gold LLT).

^{15}N concentrations were determined by mass spectrometry (ETH, Eschikon, Switzerland). For the field study we combined subsamples when individuals of the same species were growing at similar distances to the center plant.

Data analysis

We tested for effects of distance to the center and competition type on neighbour ^{33}P and ^{15}N concentrations in the glasshouse and in the field study using analysis of variance (aov function of R 3.2). When values close to zero were present, a small amount was added before log-transformation to avoid excessive leverage of these values and to achieve a normal residual distribution. Effects of distance to the center plant on log-transformed ^{33}P and ^{15}N concentrations in neighbour plants were analyzed using the model terms summarized in Table 2 and Table 3 for the glasshouse experiment and the field study respectively.

For the glasshouse experiment we included distance as continuous and factorial variable in order to account for potential non-linearity of effects. Furthermore, we included community composition as fixed term. Species was fitted within composition i.e. we treated species separately depending on the composition of the community in which they grew. For the analysis between subsequent distance zones we reduced the model terms (Table 2).

For both studies we fitted linear regressions separately for conspecific and heterospecific neighbours in order to test for distance effects in each competition type. We tested for deviation from linear relationships of isotope concentrations and distance by adding the squared distance to the original model.

Results

Glasshouse experiment

In glasshouse microcosms plant ^{15}N concentration decreased significantly with distance to the center ($F_{1,18}$= 248.7, P=0.001) (Fig. 2). Analysis of variance showed that there was a marginally significant interaction of competition type (i.e. intraspecific vs. interspecific competition) and distance effects on ^{15}N tracer concentration when distance was fitted as categorical factor ($F_{2,24}$= 3.1, P=0.06). Separate linear regressions of ^{15}N concentration in conspecific and heterospecific neighbours and their distance to the center revealed that tracer concentration in conspecifics decreased linearly with distance whereas the relationship was nonlinear for heterospecific neighbour plants (R^2=0.8, P<0.001, competition type x distance ^{2}). We analyzed effects on ^{15}N tracer concentration separately between adjacent distance zones where distance was fitted as continuous variable and found that the decrease in ^{15}N tracer concentration tended to be greater in heterospecifics than in conspecifics between 3.5 cm and 6 cm distance to the center plant ($F_{1,8}$= 4.6, p=0.05) (Fig. 2). We analyzed species compositions separately and found that the interaction of competition type and distance effects was most profound in composition 1 ($F_{2,6}$= 6.7, P=0.06, competition type x factor distance) (Fig. 2). The direction of effects did not differ between functional groups of the center plant but we found that effects were marginally significant only in pots with herbaceous center plants ($F_{2,6}$= 5.9, P=0.09, competition type x factor distance) (Table 2).

^{33}P tracer concentrations decreased significantly with distance to the center plant ($F_{1,18}$= 105, P=0.001) (Table 2) (Fig. 3). Distance effects on ^{33}P concentrations did not differ between conspecific and heterospecific individuals across or within distance intervals. Analysis of variance of each species composition showed significant interactions between competition type and distance effects on ^{33}P concentrations in species composition 2 ($F_{2,6}$= 8.6, P=0.02,

competition type x factor distance) (Fig. 3). Analysis of ^{33}P pools (data not shown) did not differ from analysis of ^{33}P concentrations.

Field experiment

In field plots ^{15}N concentration decreased significantly with distance to the center ($F_{1,12}$=595, P=0.001) (Fig. 4). The identity of the center species determined effects of distance on ^{15}N tracer concentration in conspecific and heterospecific neighbours ($F_{10,9}$=7, P=0.004, center species x competition type x distance) (Fig. 4). Analyses of ^{15}N pools (data not shown) did not differ from analysis of ^{15}N concentrations.

^{33}P tracer concentration in neighbour plants decreased significantly with distance to the center ($F_{1,12}$=52.8, P=0.001) (Fig. 5). We found that there was a significant interaction of distance to the center and competition type effects on plant ^{33}P tracer concentration ($F_{1,8}$=5, P=0.03) (Fig. 5). As opposed to ^{33}P concentrations, ^{33}P pools showed differences in effect interactions of distance and competition type between center species ($F_{10,9}$=16, P=0.01, center species x competition type x distance) (Fig. 6). Similarly, we found that the interaction of distance to the center and competition type effects on ^{33}P pools depended on the functional group of the center plant ($F_{2,9}$=4.2, P=0.05, center functional group x competition type x distance). Interactions were significant when grasses were in the center of the plot (p=0.02, $F_{1,5}$=12.4) and marginally significant when herbs were in the center ($F_{1,4}$=5.7, P=0.08) (data not shown).

Discussion

Heteromyopia is a hypothetical plant interaction mechanism which currently is solely based on theoretical assumptions (Murrell and Law 2003). We present empirical evidence from both, controlled glasshouse and natural field conditions which shows that nutrient competition distances can differ for hetero- and conspecific neighbours. The concept of heteromyopia

suggests that over short distances plants compete more intensely with heterospecific neighbours, whereas competition with conspecific neighbours is greater over long distances. Our study shows inconsistent results that do not allow us to reject the concept of heteromyopia for the nutrient uptake pattern observed for plant neighbourhoods. In the glasshouse, nitrogen (N) uptake in conspecific neighbour plants decreased linearly with distance to the center plant, whereas, in heterospecific neighbours N concentrations dropped at close proximity resulting in a nonlinear nutrient decrease with distance to the center plant. Interspecific competition for N was always greater than intraspecific competition closer to the center plant. For phosphorus (P), results were more variable but again interspecific competition tended to be greater at short distances. In the field, differences between conspecific and heterospecific neighbours were most profound for P uptake where we observed steeper decreases in nutrient uptake with distance to the center for heterospecifics. The observed differences in distance effects on nutrient uptake between conspecific and heterospecific neighbours, in the glasshouse and in the field, suggest that heteromyopia may operate through nutrient competition.

If nutrient competition should be the driver of heteromyopia, then why should belowground competition distances be greater for conspecifics when species are similar competitors? Competition for nutrients can vary depending on endogenous and external factors but generally occurs when individuals share a limiting resource from a common resource pool (Tilman 1977, Tilman and Grace 1990). Hence, the availability of a nutrient resource can determine the strength of competition for it. Further, it has been suggested that the mobility of nutrients in soils can affect the strength of plant competitive interactions as resource exploitation by competitors should be facilitated for more mobile nutrients (Huston and DeAngelis 1994, Raynaud and Leadley 2004, Wilberts et al. 2013). Differences in competition distances between con- and heterospecific neighbours, may be related to differences in nutrient mobility between them. The mobility of nutrients may differ between con- and heterospecific plants because of their integration into an arbuscular mycorrhizal fungi (AMF) network. The

association between AMF and plants can be beneficial to plant hosts in multiple ways ranging from protection from pests and pathogens (Gange and West 1994, Newsham et al. 1995) to increased nutrient uptake (Joner and Jakobsen 1994, Smith et al. 2009). AMF provide P, and to some extent N, for the plant host and receive carbon assimilates in return. P in soils is relatively immobile, organically bound, and not readily available to plants (Nye & Tinker 1977). Besides increasing the nutrient acquisition area of plants by hyphal growth (Rhodes and Gerdemann 1975, Giovannetti et al. 2001), AMF can release phosphatase and thereby access P, otherwise not plant available (Cui and Caldwell 1996). Hyphal networks establishing from different plant hosts may interconnect by hyphal fusion (anastomosis) forming a common mycorrhizal network (Newman 1988, Giovannetti et al. 2006). Host specificity of fungi is low and consequently hyphal connections can be formed between plant hosts of numerous species generating indefinitely large belowground networks across plant communities. However, the anastomosis frequency of hyphae originating from interspecific plant hosts was found to range from 44% to 49%, compared to 62% interconnectedness of hyphae between conspecific plant hosts (Giovannetti et al. 2004). This implies that nutrient mobility may differ between rhizospheres of hetero- and conspecific neighbours. In our field study, plant communities were long established and most likely well connected in AMF networks. P was transported to conspecific neighbours regardless of the distance to the center, increasing intraspecific over interspecific competition at long distances. These results might reflect a higher hyphal connectedness between conspecific plant individuals. The lack of consistent heteromyopia effects on P uptake in greenhouse microcosms may be caused by an insufficiently developed mycorrhizal network. The P measurements were carried out after 16 weeks of plant growth which may have been too short for mycorrhizal networks to establish across the microcosm. However, we found a heteromyopia effect on N uptake in plants harvested from glasshouse microcosms. N in microcosm soils may have been more mobile due to high sand content in the soil and sufficiently water-saturated pores allowing N transport via diffusion and mass flow

over long and short distances (Marschner 1986). Furthermore, plants were growing in glasshouse microcosms for 45 weeks before we measured ^{15}N tracer concentration. Low soil moisture in the field and relatively short time between isotope application and harvest may have affected the results on N transport in soils and ^{15}N tracer uptake pattern in field plant neighbourhoods.

Vogt et al. (2010) tested whether distance affected conspecific and heterospecific neighbour biomass differently. In our study, we did not grow control plants without neighbours and all species grew with con- and heterospecific neighbours. Our experimental setup does not allow comparisons between plants growing exclusively with conspecific or heterospecific neighbours. Hence, we cannot clarify whether heteromyopia effects here would have been detectable as competitive effects on plant biomass. Furthermore, we assumed that nutrient uptake in neighbour plants can be linked directly to competition with the center plant that originally received the nutrient tracer. We are aware that this is an assumption and that the nutrient was potentially not limiting for center plants. Plants growing without neighbours could show whether nutrient uptake was reduced by neighbours. However, we chose the field site based on low soil fertility and glasshouse microcosms contained a high proportion of quartz sand with low soil nutrients. Plant growth was restricted in both experimental studies, which suggests that plants were competing for nutrients.

The concept of heteromyopia was introduced as a mechanism capable of stabilizing co-existence in spatially structured communities (Murrell and Law 2003). Relatively longer interaction distances between conspecifics may create gaps for other potentially less successful species to colonize (Murrell et al. 2002). Our study revealed that nutrient uptake distances may differ between conspecific and heterospecific neighbours in controlled and natural plant communities. However, to which extent these differences contribute to the co-existence of species in plant communities remains speculative. High nutrient competition may be greater

between heterospecifics at short distances and between conspecifics at longer distances, but whether this will affect growth and survival in order for gaps to be created within the community that could be occupied by other similar competitors (Murrell et al. 2001, Rejmánek 2002) has not been tested. For future studies, we suggest a combination of nutrient competition and invasion studies using nutrient tracer in spatially structured habitats. Heteromyopia is a hypothetical concept resulting from spatial plant community patterns and the paradox of co-existing similar competitors. We found that heteromyopia may be a consequence of nutrient competition but it requires further experimental approaches and the observation of nutrient tracer movements in communities of varying spatial structure to provide empirical evidence for heteromyopia effects on plant species co-existence.

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Table 1. Number of observations from three plots for each center species. Neighbour plants were collected from increasing distances to the center plant. Distances were grouped into categories of 1-3 cm, 3-6 cm, 9-12 cm, 12-15 cm, 15-20 cm, 20-25 cm and 25-30 cm.

Center species	Prunella vulgaris									Thymus pulegioides									Bromus erectus									Festuca ovina									Helianthemum nummularium									Helicotrichon pratense								
Distance from Center (cm)	1-3	3-6	6-9	9-12	12-15	15-20	20-25	25-30	TOTAL	1-3	3-6	6-9	9-12	12-15	15-20	20-25	25-30	TOTAL	1-3	3-6	6-9	9-12	12-15	15-20	20-25	25-30	TOTAL	1-3	3-6	6-9	9-12	12-15	15-20	20-25	25-30	TOTAL	1-3	3-6	6-9	9-12	12-15	15-20	20-25	25-30	TOTAL	1-3	3-6	6-9	9-12	12-15	15-20	20-25	25-30	TOTAL
Neighbor species																																																				
P. vulgaris	2	4	3	3	1	4	6	3	26	2	2	2	1	2	6	3	4	22	1	5	1	1	3	3	2	5	21		1	2	2	2	6	5	3	21	1	2	4	3	3	2	4	5	24	1	2	5	3	2	1	4	4	22
T. pulegioides	1	3		4	4		4	2	18		3	2	1	2	3	3	1	15			1	1	1	2	5	2	12				1	1	1	2	4	9				2	1	4	2	3	12			1	1	3	2	5	4	16
B. erectus	2	5	1	2	2	5	1	4	22	2	3	1	4	2	5	3	3	23	2	3	4	1	1	2	2	4	19	1	2	2	1	1	3	3	6	19	6	2	2	1	2	4	2	4	23			3		3	5	5	4	20
F. ovina		2	3	2		3	1	4	15				3	2	2	5	2	14		2	1	3		2	4	4	16	2	1	2	1	1	3	3	1	14			1	2	1	5	3	1	13	2	1	1	2	2	7	1	3	19
H. nummularium		2	1	2	1	3	7	2	18			1	2	1	2	3	4	13			3	4	2	3		5	17		1	1	2	2	3	4	2	15		1		1		2	6	4	14		4	2	1		7		3	17
H. pratense				1	1	2		1	5				2	3	4	2		11			1	1	2	3	3	2	12				4	4	3	5	1	17		2	2	2	2	1	2	4	15	2	1	4	1	2	3	3		16

Table 2: Structure and results of analysis of variance for effects on ^{15}N and ^{33}P tracer concentrations in neighbour individuals of microcosms. Degrees of freedom for nominator (df) and denominator (ddf) may vary for analysis of seperate distance intervals, composition and functional group (FG) level. (Factor) indicates that the term "Factor Distance" was excluded for the analysis at interval level. Level of significance: n.s. = not significant, (*) =<0.1,*=0.05, **=0.01, ***=0.001.

Level	Term	df	Error	ddf	^{15}N (µg per g biomass) (log)									^{33}P (Bq per g biomass) (log)								
					all	By distance			By composition[1]			By FG		all	By distance			By composition[1]			By FG	
						3.5-6 cm	6-7 cm	7-9 cm	Comp 1	Comp 2	Comp 3	Grasses	Herbs		3.5-6 cm	6-7 cm	7-9 cm	Comp 1	Comp 2	Comp 3	Grasses	Herbs
Pot																						
	Center species	4	Pot	2	(*)	n.s.	(*)	n.s.	–	–	–	–	–	n.s.	n.s.	n.s.	n.s.	–	–	–	–	–
	Composition	2	Pot	2	(*)	n.s.	n.s.	n.s.	–	–	–	–	–	n.s.	n.s.	n.s.	n.s.	–	–	–	–	–
Pot x Competition type																						
	Competition type	1	Pot x Competition type	8	n.s.	n.s.	(*)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Pot x (Factor) Distance																						
	Distance	1	Pot x (Factor) Distance	18	***	***	**	*	*	***	***	**	***	***	**	**	n.s.	***	**	***	***	***
	Factor Distance	2	Pot x Factor Distance	18	***	–	–	–	n.s.	*	**	n.s.	*	**	–	–	–	n.s.	n.s.	(*)	**	**
Pot x Competition type x (Factor) Distance																						
	Competition type x Distance	1	Pot x Competition type x (Factor) Distance	24	n.s.	(*)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Competition type x Factor Distance	2	Pot x Competition type x Factor Distance	24	(*)	–	–	–	(*)	n.s.	n.s.	n.s.	(*)	n.s.	–	–	–	n.s.	*	n.s.	n.s.	n.s.
Pot x Neighbor species x Composition																						
	Neighbor species x Composition	6	Pot x Neighbor species x Composition	3	*	n.s.	n.s.	n.s.	–	–	–	–	–	(*)	n.s.	n.s.	(*)	–	–	–	–	–

Table 3: Structure and results of analysis of variances for effects on ^{15}N and ^{33}P tracer concentration and ^{33}P tracer pools in neighbour individuals of field plots and separately for plots of each center species. Degrees of freedom for nominator (df) and denominator (ddf) may vary for analysis by center species and functional group (FG). Level of significance: n.s. = not significant, (*) =<0.1*=0.05, **=0.01, ***=0.001.

Level	Term	df	Error	ddf	^{15}N (µg per g biomass) (log+0.2)							^{33}P (Bq per g biomass) (log+3.5)	^{33}P (Bq per plant) (log+0.5)							By FG	
					all plots	B. erectus	F. ovina	H. Nummularium	H.pratense	P. vulgaris	T. pulegioides	all plots	all plots	B. erectus	F. ovina	H. nummularium	H.pratense	P. vulgaris	T. pulegioides	Grasses	Herbs
Plot																					
	Center species	5	Plot	12	(*)	–	–	–	–	–	–	(*)	n.s.	–	–	–	–	–	–	n.s.	(*)
Plot x Competition type																					
	Competition type	1	Plot x Competition type	9	(*)	*	**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	–	n.s.	n.s.	*	n.s.	n.s.
Plot x Distance																					
	Distance from Center	1	Plot x Distance	12	***	***	(*)	**	*	**	**	***	***	n.s.	*	–	n.s.	*	**	**	**
Plot x Competition type x Distance																					
	Competition type x Distance	1	Plot x Competition type x (Distance)	6	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.	*	**	n.s.	n.s.	–	n.s.	n.s.	(*)	*	(*)
	Center species x Competition type x Distance	7	Plot x Competition type x (Distance)	6	**	–	–	–	–	–	–	n.s.	*	–	–	–	–	–	–	(*)	(*)
Plot x Neighbor species																					
	Neighbor species	5	Plot x Neighbor species	53	***	(*)	n.s.	*	n.s.	n.s.	(*)	(*)	*	n.s.	n.s.	–	n.s.	(*)	n.s.	n.s.	*
	Neighbor species x Competition type	5	Plot x Competition type	9	n.s.	–	–	–	–	–	–	*	n.s.	–	–	–	–	–	–	n.s.	n.s.

Figure 1: Arrangement of plant individuals in experimental microcosms. Three colours represent three different species.

Figure 2: Mean ^{15}N tracer concentration in conspecific (dashed line) and heterospecific neighbours (solid line) at each distance across microcosms and for each species composition separately (Comp 1= *F. pratensis, H. pilosella, P. lanceolata*; Comp 2= *H. lanatus, P. lanceolata, P. media*; Comp 3= *F. pratensis, H. lanatus, P. lanceolata*). Error bars show the standard error of the mean. ^{15}N data here and for analysis was log transformed for normal residual distribution. The axis shows the corresponding untransformed values.

Figure 3: Mean ^{33}P tracer concentration in conspecific (dashed line) and heterospecific neighbours (solid line) at each distance across microcosms and for each species composition separately (Comp 1= *F. pratensis, H. pilosella, P. lanceolata*; Comp 2= *H. lanatus, P. lanceolata, P. media;* Comp 3= *F. pratensis, H. lanatus, P. lanceolata*). Error bars show the standard error of the mean. ^{33}P data here and for analysis was log transformed for normal residual distribution. The axis shows the corresponding untransformed values.

Figure 4: ^{15}N tracer concentration and distance to the center plant for conspecific (empty circles) and heterospecific (filled circles) neighbour plants in all field plots and in plots for each center plant species separately. Lines show linear regression for conspecific (dashed line) and heterospecific neighbours (solid line). ^{15}N data here and for analysis was log +0.2 transformed for normal residual distribution. The axis shows the corresponding untransformed values.

Figure 5: ^{33}P tracer concentration and distance to the center plant for conspecific (empty circles) and heterospecific (filled circles) neighbour plants in all field plots. Lines show linear regression for conspecific (dashed line) and heterospecific neighbours (solid line). ^{33}P data here and for analysis was log +3.5 transformed for normal residual distribution. The axis shows the corresponding untransformed values.

Figure 6: ^{33}P tracer pools and distance to the center plant for conspecific (empty circles) and heterospecific (filled circles) neighbour plants in all field plots and in plots for each center plant species separately. Lines show linear regression for conspecific (dashed line) and heterospecific neighbours (solid line). ^{33}P data here and for analysis was log +0.5 transformed for normal residual distribution. The axis shows the neighbour corresponding untransformed values

Figure 1

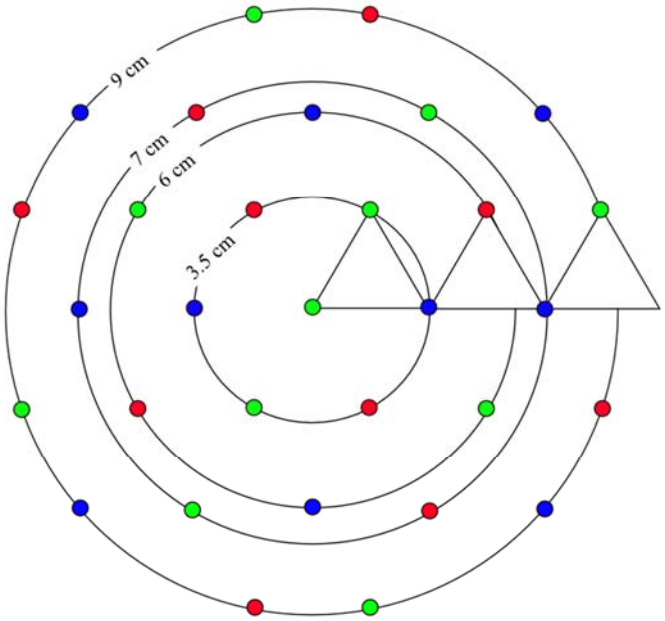


Figure 2

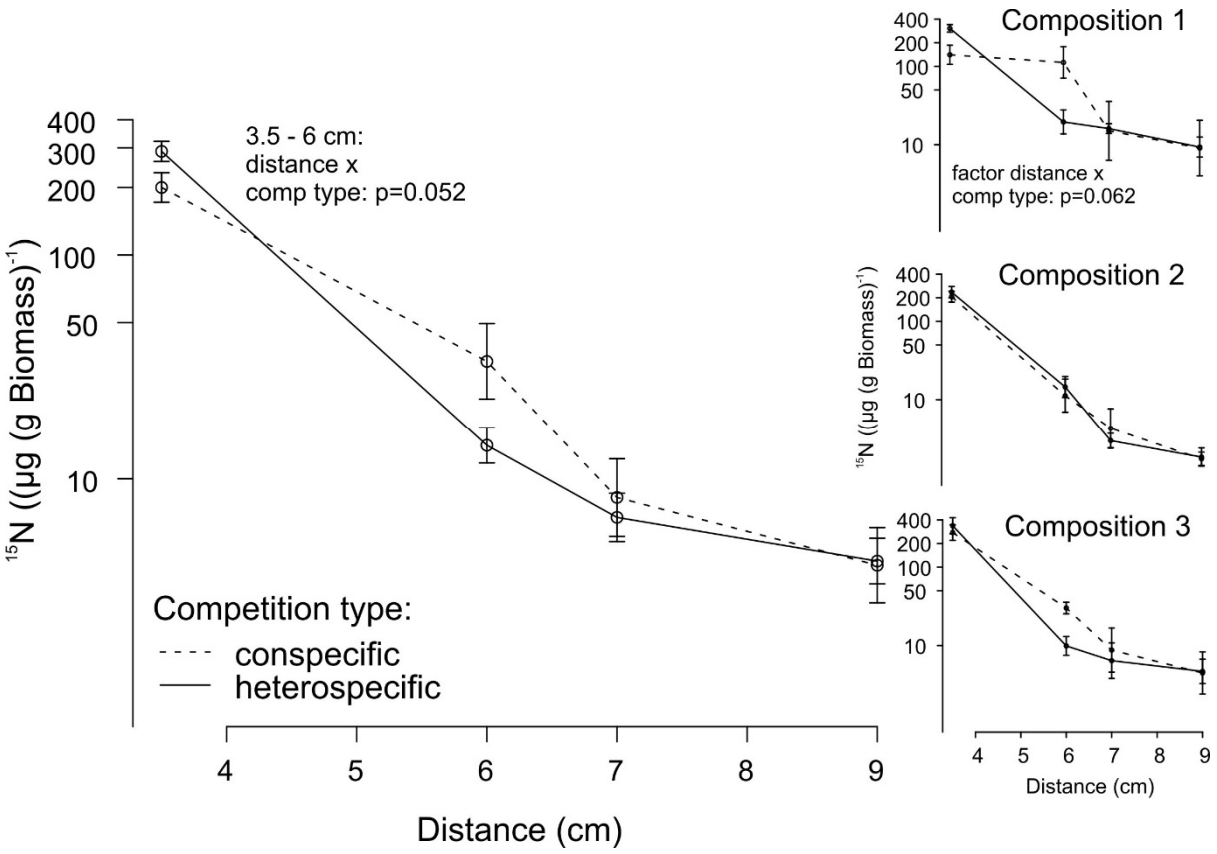

Composition 1
Composition 2
Composition 3
3.5 - 6 cm:
distance x
comp type: p=0.052
factor distance x
comp type: p=0.062
^{15}N ((µg (g Biomass)$^{-1}$)
Distance (cm)
Competition type:
conspecific
heterospecific

Figure 3

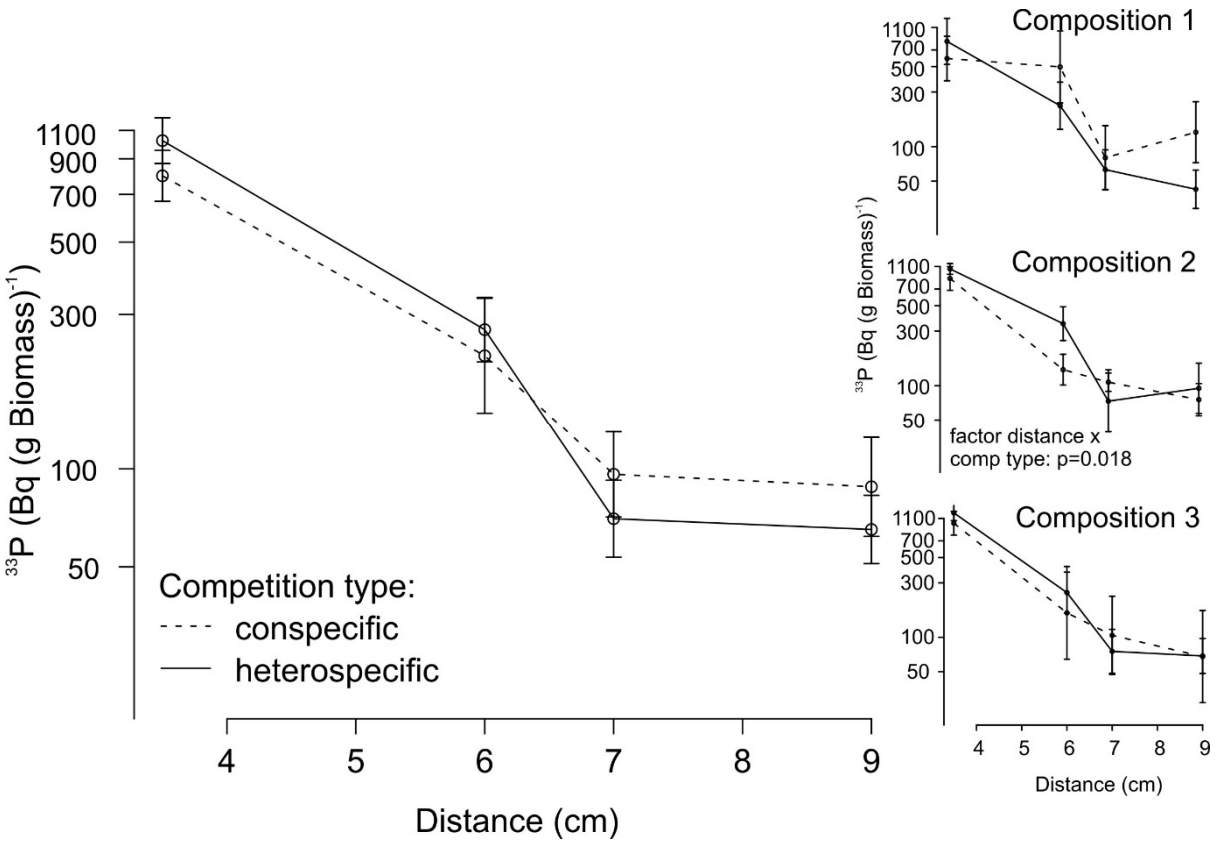

Composition 1
Composition 2
Composition 3
factor distance x
comp type: p=0.018
Competition type:
conspecific
heterospecific
^{33}P (Bq (g Biomass)$^{-1}$)
Distance (cm)

Figure 4

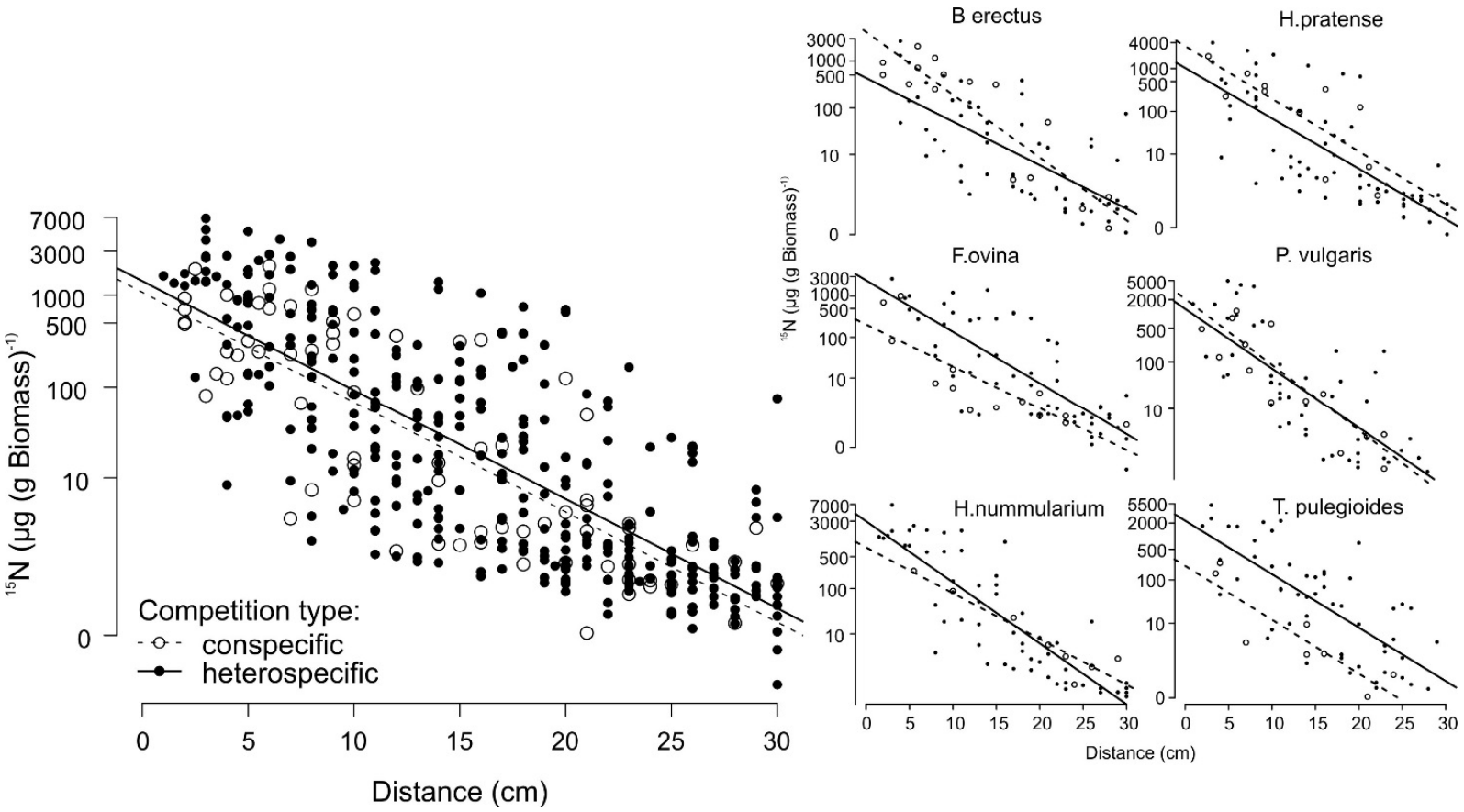

B erectus
H.pratense
F.ovina
P. vulgaris
H.nummularium
T. pulegioides
^{15}N (µg (g Biomass)$^{-1}$)
Distance (cm)
Competition type:
conspecific
heterospecific

Figure 5

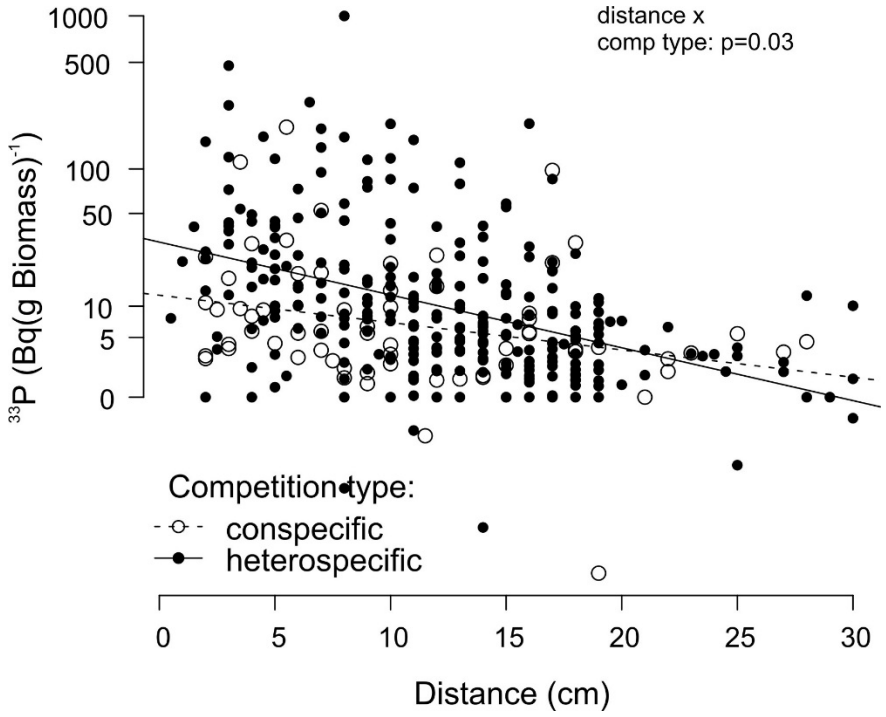
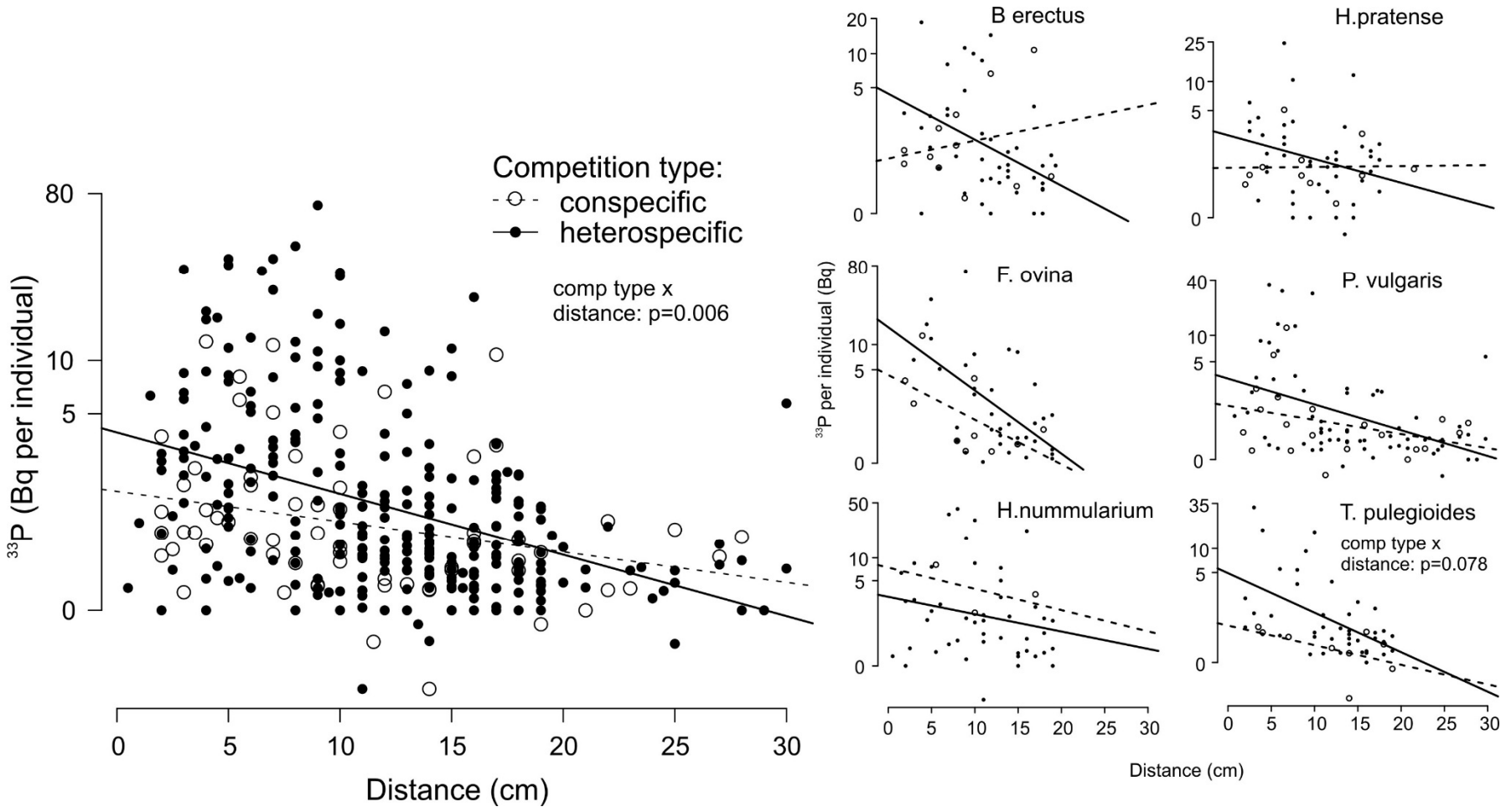

distance x
comp type: p=0.03
1000
500
100
50
10
5
0
^{33}P (Bq(g Biomass)$^{-1}$)
Competition type:
conspecific
heterospecific
0
5
10
15
20
25
30
Distance (cm)

Figure 6


Competition type:
conspecific
heterospecific
comp type x
distance: p=0.006
^{33}P (Bq per individual)
Distance (cm)
B erectus
H.pratense
F. ovina
P. vulgaris
H.nummularium
T. pulegioides
comp type x
distance: p=0.078
^{33}P per individual (Bq)
Distance (cm)

Discussion

General Discussion

The recent loss of biodiversity across ecosystems and the associated consequences of that loss for humanity have motivated research on the driving mechanisms of species co-existence and biodiversity conservation for almost three decades (Isbell et al. 2011, Cardinale et al. 2012, Hooper et al. 2012, Naeem et al. 2012, Rio de Janeiro Earth Summit 1992). Competition within plant communities is widely accepted as major determinant of plant species co-existence (Gause 1934) and community diversity. However, the mechanisms affecting plant competitive outcome are still under debate. In this dissertation, I focused on two factors that may affect plant competitive interactions and species co-existence; nutrient mobility and the distance-dependency of plant competitive interactions. The results of my study contribute towards a greater understanding of plant competitive interactions.

According to Tilman's (1977) resource-partitioning theory, organisms within a community partition resources to reduce competition for the same resources. When concentration equilibrium has been reached within the community, the trade-off between minimum resource requirements of those species determines their co-existence. However, the quantity of nutrients depleted from a resource pool in the soil is dependent on the ability of nutrients to move towards roots (Bray 1954) and the degree of soil nutrient mobility may affect competitive interactions and species coexistence within plant communities (Huston and DeAngelis 1994). Plants pre-empt resources along their root surface and thereby create concentration gradients in the soil rather than reducing concentrations across a resource pool homogenously (Craine et al. 2005). High nutrient mobility allows nutrient transport along concentration gradients, consequently enabling plant individuals to reduce the resources within a resource pool more effectively and therefore increase their competitiveness with other plants in the community. This theoretical assumption was important when included in modelling approaches (Huston and DeAngelis 1994, Raynaud and Leadley 2004, Raynaud et al. 2008) and when tested empirically (Wilberts

et al. 2013). Studies by Wilberts et al. (2013) showed that the manipulation of nutrient mass flow and associated nutrient mobility promoted intraspecific competition as indicated by greater size differences between small and large plants. Such an increase in size difference in response to nutrient mobility was confirmed in a further study by Wilberts et al. (2013) in which nutrient transport was manipulated with the insertion of barriers with increasing openness between plants. The focus of this dissertation was to empirically explore the effects of nutrient mobility on plant competitive interactions. The study of such a potentially important determinant of resource competition in terrestrial plant communities may further our understanding of plant species co-existence and community structure. In this study, soil nutrient mobility was manipulated with both abiotic mechanisms (experiment 1) and biotic interactions (experiment 2) providing further insights into the effects of nutrient mobility on plant competition and consequently for plant community structure.

In **Chapter 1** I found that increased nutrient supply at reduced nutrient mobility had a greater effect on the outcome of competitive interactions than reduced nutrient mobility per se. In contrast to the study by Wilberts et al (2013) in which high nutrient supply increased the effects of high nutrient mobility on competition, I found that nutrient mobility was less important than previously suggested. On the contrary, Huston and DeAngelis (1994) proposed that high nutrient mobility and low supply rates will result in the greatest competitive effects. In my study, the addition of the nitrification inhibitor and reduction of nutrient mobility increased the nutrient supply for plants, potentially increasing competition between them. I suggest the effects of sufficient/increased nutrient supply on plant competitive interactions may have counteracted the potential effects of decreased nutrient mobility.

The role of nutrient supply in competitive interactions

The role of soil fertility and nutrient supply in determining competitive interactions between plants has been subject of heated debate (Thompson 1987, Tilman 1987a, 1989,

Thompson and Grime 1988, Grace 1991). There are two opposing views on the effects of soil fertility on competitive interactions. Grime (1979, 1987) suggested nutrient-poor environments act as a stressor on plant communities preventing potentially strong competitors from dominating the community, with species more able to tolerate stress becoming more abundant. Competition is therefore less important with a decrease in soil fertility because "good competitors" cannot dominate the community. Tilman (1982, 1988) in contrast, proposed competition can be just as intense in nutrient poor environments as it is in productive environments although the importance of belowground and aboveground competition may vary. Grace (1991) states what these opposing views have in common is, at low productivity or in high stress situations, selection is for species that tolerate low resource supply rates. A further similarity between Tilman's and Grime's theories is the confusion of the terms intensity of competition and importance of competition. Whereas competition intensity describes the effect competition on an organism expressed for example as biomass reduction, importance of competition describes those effects in relation to other growth limiting factors (Grace 1991, Brooker et al. 2005). Grime's focus is generally on the importance of competition. Tilman in contrast often refers to the intensity of competition when comparing competition along nutrient gradients and to importance of competition when he discusses aboveground and belowground competition. In general, high nutrient availability increases plant growth and therefore aboveground competition is considered more important in high productive environments (Wilson and Tilman 1991, 1993, 1995, Putz and Canham 1992, Hautier et al. 2009). In this study I found that aboveground growth was increased by greater nutrient availability and although I excluded aboveground competition, our results hint at increased success for good competitors contributing to the increasing evidence (Wilberts et al. 2013) that belowground competition may be just as important as aboveground competition under more productive conditions.

For this study it was essential that there was belowground competition to test the potential reduction in competitive interactions with low nutrient mobility. I found that plants grown in pots without competition produced significantly more biomass than plants grown in communities suggesting that competition was important for individual plant growth.

Nutrient supply is clearly important for the outcome of competitive interactions between plants. In contrast to the study by Huston & DeAngelis (1994) and similarly to the study by Wilberts et al. (2013), our results suggest high nutrient supply increases the importance of competition. Here the increased importance of competition potentially outweighed any mitigating effects of nutrient mobility.

The role of CMN in competitive interactions

Arbuscular mycorrhizal fungi can develope large hyphae systems connecting plants into a common mycorrhizal network (CMN) and as the fungi have little host specificity, plants of different species can be connected in the same hyphal networks (Newman 1988, Giovannetti et al. 2006). To which extent different plant hosts invest into the symbiosis within CMNs and whether this drives the benefit they derive from the association, is still not fully understood (Lekberg et al. 2010, Fellbaum et al. 2014) but implies plant interactions may be affected.

In **Chapter 2** the integration of plants into a CMN was shown to promote plant productivity and facilitate complementarity between plant individuals within a community. The symbiosis between fungi and plants is generally thought to be mutualistic, with both symbiotic partners benefiting from the association. However, the benefits of arbuscular mycorrhizal fungi (AMF) colonisation for plants were found to be species specific, ranging from highly beneficial to antagonistic associations (Kiers and van der Heijden 2006, Wagg et al. 2015). In addition, environmental differences and AMF community species composition and richness may affect the outcome of plant associations with AMF (van der Heijden et al. 1998). Species specific

responses to AMF colonisation implies symbiosis with AMF may be of great importance for plant competitive interactions and species coexistence.

Experimental studies have demonstrated that in plant communities of different species, the presence of AMF fungi in the soil could determine the dominance of plant species in the community dependant on their response to AMF (Grime et al. 1987, Hartnett et al. 1993, Zobel and Moora 1995, Moora and Zobel 1996, West 1996, Scheublin et al. 2007, Wagg et al. 2011).

AMF associations generally relax plant competitive interactions if the inferior plant host is favored by the symbiosis (Grime et al. 1987, Urcelay and Díaz 2003, van der Heijden and Horton 2009, Wagg et al. 2011) or if the growth of inferior plants is facilitated in other ways (ref). It has been suggested that some plants may maintain hyphal networks by investing relatively more assimilates which would indirectly benefit other plants that invest fewer assimilates (Walder et al. 2012). Similarly, it has been suggested that photosynthetic assimilates may be transferred between plants through hyphal networks by which plants would facilitate others directly as carbon donor (Grime et al. 1987, Read 1997, Simard and Perry 1997). However, experimental studies have demonstrated that assimilates, although transferred into other roots, generally remain in fungal tissue and therefore do not benefit the host directly (Watkins et al. 1996, Graves et al. 1997, Robinson and Fitter 1999).

Both the presence and absence of AMF and the integration or disconnection of plants from a CMN, affected plant competitive outcome (Weremijewicz and Janos 2012). Weremijewicz & Janos (2012) demonstrated that integration in a CMN increased size inequality in monoculture seedling stands indicating that intraspecific competition was increased by a CMN.

In this study, I did not find that size inequality in monocultures decreased with the disconnection from a CMN but rather I found complementarity between species in mixtures was enhanced. The importance of CMN connectivity for individual growth (see paragraph

about nutrient supply and competition) and complementarity of the community potentially may have masked the effects of nutrient competition due to reduced nutrient mobility.

In **Chapter 1** and **Chapter 2**, I tested the the effects of nutrient mobility on plant competition**.** I found the possible negative effects of reduced soil nutrient mobility on competitive interactions may have been confounded by the effects of increased nutrient supply or facilitation. The results of these studies however provide interesting insights into the mechanisms behind plant competitive interactions. Nutrient mobility did not affect competition between plants as has been shown previously (Wilberts et al. 2013) and the presented studies do not confirm Huston & DeAngelis' (1994) theory. Nutrient supply needs to be considered when analysing competitive effects related to nutrient mobility as stated by Huston and DeAngelis (1994). Our studies provide empirical evidence and support previous results (Wilberts et al. 2013) that high nutrient supply increases belowground competition.

In future studies, I suggest an experimental setup that tests the effects of nutrient mobility along gradients of nutrient supply rates removing confounding effects. Mass flow rates could be manipulated by varying the grain size of a sand substrate. This way the mass flow would be affected by increasing pore sizes in the soil without creating physical barriers between plants which I think is necessary in order to allow unrestricted root growth and therefore competition between plants. A factorial fertilizer treatment would test the relationship of nutrient supply rates and nutrient mobility effects on plant competition.

The role of space in plant competitive interactions

The interaction of plant individuals is generally restricted to their immediate neighbourhood as they are sessile (Vogt et al. 2010). Limited seed dispersal and clonal growth create spatially structured habitats that distinguish plant communities significantly from animal communities. Whereas animal communities can be viewed as randomly distributed, plant

communities commonly exist in conspecific clusters (Stoll and Weiner 2000). The relative importance of intraspecific competition over interspecific competition is therefore greater in plant communities. Considering space in plant interactions is crucial, as has been demonstrated in empirical (Tilman 1994, Rees et al. 1996, Milbau et al. 2007) and theoretical studies (Tilman and Kareiva 1997, Amarasekare 2003, Turnbull et al. 2007) that contributed to the advancing field of spatial plant ecology. Spatial aggregation in plant communities is suggested to promote species co-existence as interactions with conspecifics protects inferior competitors from the higher competitive strength of superior heterospecifics (Weiner and Conte 1981, Rees et al. 1996, Murrell et al. 2001, Stoll and Prati 2001, Monzeglio and Stoll 2005, Mokany et al. 2008, Wassmuth et al. 2008, Porensky et al. 2012). At the edge of clusters, however, superior competitors can outcompete inferior species and eventually exclude them (Chesson and Neuhauser 2002). Spatial aggregation cannot therefore foster stable co-existence. Niche theory implies that habitat heterogeneity promotes co-existence (Barot and Gignoux 2004). Plants can create habitat heterogeneity for example by pre-empting resources (Barot and Gignoux 2004). This form of heterogeneity is based on plant properties and therefore endogenous. A niche created around a plant may be more easily occupied by conspecifics than by heterospecifics dependent on that individual's competitive effect on other plants. Murrell & Law (2003) suggest that competition between conspecifics may be greater over long distances whereas interspecific competition is greater in the immediate neighbourhood. If plants compete more strongly with their own kind over long distances than with other species, strong competitors exclude themselves over long distances and thereby create gaps which could then be occupied by other plant individuals. Mechanisms that create holes generally lead to stable coexistence (Barot and Gignoux 2004). Heteromyopia was suggested to stabilise co-existence in spatially structured plant communities (Murrell et al. 2002, Murrell and Law 2003). Based on the nutrient uptake of the plant species in this study, I could show that competitive interaction distances may differ between conspecific and heterospecific neighbours (**Chapter 3**). The strong effects of

phosphorous (P) uptake in the field suggest that high intraspecific competition over longer distances may be based on the connection of conspecifics in a common mycorrhizal network. Weremijewicz & Janos (2012) have shown that intraspecific competition was mediated by the connectivity of plants in CMNs. The study presented here was a novel approach to uncover potential drivers of interaction distance differentiation, further empirical tests will be needed to support these findings.

The implications of heteromyopia have been debated (Murrell et al. 2002, Rejmánek 2002) and it was suggested that the differentiation of interaction distances between hetero- and conspecifics could stabilize coexistence in spatially structured habitats. Although this study demonstrates that the underlying mechanism may be based on nutrient competition between plants, I cannot conclusively determine such effects on plant community structure. In order to show that heteromyopia can stabilize coexistence, further empirical studies are required to explore the effects of heteromyopia on invasion and survival success in spatially structured plant communities.

Studying plant competition

The experiments presented in this thesis illustrate the difficulties associated with the study of plant competitive interactions. Competition can be defined as "The negative effects that one organism has upon another by consuming or controlling access to, a resource that is limited in availability" (Keddy 2001). But what negative effects does competition have on plants? In a perfect environment, in which no resources are limited by environmental constraints or by competition, biomass production and reproductive success could reach an individual's inherent limit. Divergence from this maximum performance is generally interpreted as negative effect. Biomass production and reproductive success are the consequence of the negative effects of plant competition although not the negative effect itself. Plant biomass production is affected

by numerous biotic and abiotic factors such as temperature, soil resources, pollinators, herbivores, parasites, mutualistic partners and competition.

Studying competition in plants is challenging as biomass reductions caused by competition have to be separated from other growth limiting factors. Many competition experiments are highly debated because such factors are ignored, or treated as minor side effects, and biomass reductions are directly interpreted as competitive effects (Trinder et al. 2013). Direct measurements of competition are rare and have to happen at the resource level; as an example the reduced amount of light reaching an individual through shading by neighbours can be directly measured (Wilson and Tilman 1993, Hautier et al. 2009). For belowground resources, the reduction in resource use by one plant due to the presence of another is technically more difficult to measure. Belowground resources that are a potential source of competition for plants are space, water, symbionts and a variety of macro-and micronutrients, of which nitrogen is commonly the most limiting in natural ecosystems (Tilman 1987b). Isotopic labelling techniques allow direct measurements of nutrient fluxes in soils and facilitate the interpretation of negative neighbour effects in competition experiments (Trinder et al. 2013). Here, I used isotopic labelling techniques in order to meet the increasing demand for more direct measurements of competition (Trinder et al. 2013). In my present study, although I measured nutrient uptake of plants from the nutrient acquisition area of neighbour plants, interpretation of the results was not clear. I made the assumption that nutrient tracer applied in the acquisition area of a plant would beused by that particular plant in the absence of competition. I therefore assumed that there was competition for that resource and interpreted any uptake by neighbours as competitive strength. I cannot exclude however that the resource was not limited and use by neighbours potentially not harmful to the focus individual.

Competition studies are often criticised because not only are results obtained from biomass measurements ambiguous, but they are generally measured at the end of an experiment

(Trinder et al. 2013). Time was found to be an important factor for plant competitive interactions, especially in natural communities where plants of different ages grow together. At the seedling stage competition can be greater if neighbouring individuals are larger (Ramseier and Weiner 2006) however competition may decrease once plants reach similar sizes. I monitored plant growth over the course of the experiment (Chapter 1 and 2) in order to capture potential phases of high or low competition. In both studies, I could demonstrate that growth curves of competition-free controls and 4 quadrant communities diverged after approximately two weeks. I did not find that the intensity of competition changed significantly after two weeks.

Plants in monocultures have similar competitive abilities and generally it would be advisable to produce a size or age gradient linked with a competition gradient (Ramseier and Weiner 2006). In my experiment, I planted seedlings at similar times and therefore created equal chances for establishment and competitive strength. As I aimed to analyse species richness effects, I grew monocultures of equal age for accurate comparisons with other communities.

Conclusion

The results presented in this thesis contribute to our understanding of plant competitive interactions. I did not find that nutrient mobility was important for plant competitive outcome in this study. The positive/negative effects of nutrient supply and facilitation in CMNs determined and highlighted their importance for plant competitive outcome. I could show that differentiation of interaction distances can occur under controlled glasshouse and field conditions which has implications for plant species coexistence. Experimental approaches as presented in this thesis contribute to methodological advances that further our ability to affect, quantify and qualify competition and therefore contribute to a greater understanding of competition driving mechanisms.

The knowledge of mechanisms that drive competitive interactions is of major importance for our understanding of plant community ecology. The interactions we observe at greater scales, such as biodiversity effects, originate from small scale interactions between single individuals. Bottom-up approaches in biodiversity research further our understanding of underlying processes and contribute to our ability to explain, protect and potentially restore degraded ecosystems.

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Acknowledgements

I want to thank my PhD committee and advisors Pascal A. Niklaus, Bernhard Schmid, Peter Stoll and Emmanuel Frossard for their support, expertise and advice.

I want to thank Andrea-Pfisterer and Luisa Last in their role as coordinators of the PhD program "Plant Sciences and Policy" and their administrative and moral support and Isabel Schöchli and Lilian Dutoit because nothing would have worked without you and your patience!

Thanks to Andreas Stampfli from University of Bern for providing help and information regarding my field studies in Thun and Rolf Daenzer from the military site in Thun for the support on site.

Thanks to Kurt Bösiger and his colleagues at the Physik-Institut for their creative input to our plant pot design and a memorable aperó!

Thanks Federica Tamburini from ETH Eschikon for all the lab work!

Special Thanks goes to everyone who was directly involved in helping me in the greenhouse, the lab, the field and behind the desk. Theres Zwimpfer, Matthias Furler, Anna Kolly, Juliana Nates, Lukas von Känel, Saeed Karbin, Adele Ferrari, Enrica De Luca, Daniel Trujillo, Dorde Topalovic, Dzaeman Dzulkifli, Elvira Schnyder, Nadia Castro, Katherine Horgan, Debra Zuppinger-Dingley, Michael O'Brien, Cameron Wagg, Rich Baxter.

And a very special Thanks goes to Rene Husi who helped and supported me in the lab and made my days in rooms without windows worthwhile.

Four years have past and I was always surrounded by the most wonderful people. Rich, Katie, Claire, Juliette, Enrica, Debra, Matthias, Dzaeman, Gian-Marco, Mollie, Sam. You made me happy, and supported; even carried me for a bit of the way. There are no words as big as my gratitude. You will always be with me!

And finally I want to thank my family for making me feel home, wherever I am. I could not wish for more love, faith and support. Danke!

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